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Preface & Acknowledgements

These are the extended abstracts for the keynote lectures, special symposia and technical sessions of the 5th International Conference on the Biogeochemistry of Trace Elements held at the Technical University Vienna, July 11-15, 1999.

This conference was held in continuation of a highly successful conference series that was started in 1990 in Orlando/Florida, with follow-ups in Taipei/Taiwan (1993), Paris/France (1995), and Berkeley/California (1997). At the time the proceedings went to press we expected 450 to 500 participants from 50 countries. This represents clearly an increase in attendance, making this conference series a prinicipal one among international meetings on the biogeochamistry of trace elements.

The conference is dedicated to explore and discuss contemporary and emerging issues in biogeochemistry research of trace elements. It provides a forum for professionals, regulators, and students to present their most recent findings and to discuss with colleagues from around the world the state-of-the-art in methodology, analytical techniques, and process development.

Conference topics cover important aspects of fundamental research such as kinetics and mechanisms of the fate of trace elements, including radionuclides, in soils and related ecosystems and methods for their assessment.

Based on the numerous contributions on the bioavailability of trace elements, this topic was chosen as a general conference theme. The keynote lectures, delivered by L. Kochian and I. Thornton, addressed complementary aspects, i.e. plant-soil interactions focussing on the rhizosphere, and the foodchain.

The 5th ICOBTE '99 included ten special symposia, coordinated by M. Mench, emphasizing key areas in trace element research, organized by experts in the respective topics as indicated below:

- S1 Phytoremediation – A.J.M. Baker, D. Salt, J. van Vangronsveld
- S2 Fate of Radionuclides –F. Carini, P. Coughtrey, C. Bunnenberg, M. Gerzabek
- S3 Trace Elements and Pedology – D. Baize, J.-C. Vedy
- S4 Bioavailability, Fluxes and Transfer of Trace Elements in Soils and Soil Components - M.B. Kirkham, I.K. Iskandar, A. Banin
- S5 Fate of Trace Elements in the Rhizosphere – G. Gobran, E. Lombi, W.W. Wenzel
- S6 Remediation of Metal-Contaminated Soils – R. Naidu, N.W. Lepp, N. van der Lelie
- S7 Trace Element Issues in Developing Countries – Z.-S. Chen, D. Chakraborti
- S8 Trace Elements in Biosolids and Wastes Applied to Land – S.P. McGrath, P. Sequi, F. Zhao
- S9 Kinetics and Mechanisms of Trace Element Sorption/Release on Natural Materials – M. Selim, D.L. Sparks
- S10 Metal-Organic Interactions – R.S. Sletten, N. Senesi

In addition, nineteen technical sessions were arranged from the volunteered contributions by the editors of this exteded abstract book.

The review process was coordinated by the editors. Manuscripts in the proceedings were peer reviewed by the members of the various committees of ICOBTE '99 and symposia organizers. The organizers wish to acknowledge with thanks, the peer reviews provided by these colleagues. We also wish to express our thanks to those who were involved in technical

aspects of the review process and in editing the proceedings: A. Schnepf, N. Kirchbaumer, D. Schnepf, M. Schweiger and S. Strasser.

The conference was supported by the University of Agricultural Sciences Vienna and the International Union of Soil Science. Main sponsorship was provided by The City of Vienna, including the Office of the Mayor and Governor and the Department MA 18 (Group Science), the Austrian Federal Ministry of Science and Transport, and the American Society of Soil Science. Additional sponsoring came from The Vienna Convention Bureau, the European Research Board Office of the U.S. Department of Defense and the U.S. Department of Energy through the Savannah River Ecology Laboratory, the National Taiwan University, Die Burgenländische Anlage & Kreditbank AG., and Fürst Esterházy'sche Privatstiftung Lockenhaus.

On behalf of the editors:

Walter W. Wenzel

A TRIBUTE TO

Harry Verney Warren

OC, OBC, B.A.Sc., B.A., M.Sc., D. Phil (Oxon), D.Sc. (Hon. Waterloo, UBC), FRSC, FGSA

August 27, 1904 – March 14, 1998

Last year saw the passing of one of the great pioneers in the biogeochemistry of trace elements, Professor Harry Warren. Harry Warren was associated with the University of British Columbia (UBC) in Canada for more than 75 years; first as a student (1921) obtaining his BA in 1926 and a Bachelor of Applied Science degree the following year. After a few years gaining post graduate degrees at Oxford University (UK) he joined the faculty of the Department of Geology and Geography at UBC in 1932 where he was a professor until his retirement in 1973. For the next 20 years he remained as active as ever in his role as emeritus professor.

During his undergraduate years Harry Warren became involved in a wide range of extracurricular activities, notably sports and acting. He helped form the UBC field hockey club and the Vancouver junior cricket league, and played on the senior rugby team. However, it was in track events that he truly excelled, winning the 100, 220 and 440-yard dashes at UBC in 1924. Later, whilst a British Columbia Rhodes Scholar at Queen's College, Oxford (1926-1929), he was awarded his Blue, won the 220-yard dash at the British Games in London in 1927, and was a member of the Canadian team at the 1928 Olympic Games in Amsterdam. The same year he equalled the world record in the 100-yard dash on grass at the Irish Games meeting in Dublin.

Harry Warren is renowned as the 'Father of Biogeochemical Prospecting' and was a pioneer in the application of the biogeochemistry of trace elements to exploration, environmental studies, and relationships to the health of humans and quality of agricultural products. Many of his 197 publications deal with the relationship between the metal content of the ground (rock, soil and water) and that of trees and shrubs. These investigations were applied to the exploration for metal deposits (notably Cu, Zn, Pb, Mo, Ni, Au, Ag, Hg) and to environmental studies (Pb, Ag, Hg, As), and remain the fundamental building blocks for current developments in this intriguing multi-disciplinary and burgeoning field of endeavour.

Amongst his varied geological interests, of which most were related to trace element geochemistry of a comprehensive range of surface materials, he became increasingly involved in the relationships between metal concentrations and health problems. As early as 1954 he published a paper entitled 'Geology and Health', and in 1963 the Journal of the Royal College of General Practitioners published his paper entitled 'Trace Elements and Epidemiology'. He went on to study possible relationships between trace metals and the incidence of cancer and multiple sclerosis. His efforts in this area of what he sometimes referred to as 'Medical Geology' were formally recognised in 1973 when he was installed by Prince Philip as an Honorary Fellow of the Royal College of General Practitioners in England. His last published paper (1989) was entitled "Geology, Trace Elements and Health'.

Harry Warren was a founder member of the Association of Exploration Geochemists and the Society of Environmental Geochemistry and Health. In addition to his pioneering research activities, he provided inspiration to generations of students, and acted as a consultant on mineral exploration techniques to the mining industry. For

58 years he taught a prospecting school for the British Columbia and Yukon Chamber of Mines, and for a period lectured prison inmates on prospecting and mineral identification.

Harry Warren has aptly been described as a lateral thinker, ahead of his time. His broad-ranging, fertile and innovative mind led to a range of rather exotic tests, such as the analysis of bee pollen and trout livers to assist in locating mineral-rich areas, and the use of dogs in the bush to sniff out sulphide-rich boulders. These studies have been the inspiration for others to continue to build on the foundation of biogeochemical observations and information that he laid, using the fundamental advances in low cost multi-element analytical technology that have emerged in recent years.

For the wealth of information that Dr. Warren produced, and his efforts in so many areas, he has been awarded many distinctions. He was made a Fellow of the Royal Society of Canada in 1945. In 1971 he was made an Officer of the Order of Canada and in 1991 he received the Order of British Columbia. In 1990 he was inducted into the British Columbia Sports Hall of Fame for his tireless efforts to promote field hockey, and into the UBC Sports Hall of Fame in 1993. On 20 January of this year he was inducted into the Canadian Mining Hall of Fame.

I did not meet Harry Warren until about twenty years ago, but in my many subsequent meetings with him I could certainly relate to what others have described as his >boundless energy= and his >unbridled enthusiasm=. In the eulogy at his funeral it was noted that he never lost his inherent graciousness and mischievous twinkle. In addition to his achievements in sports this remarkable man has had a great influence on the development of the biogeochemistry of trace elements, environmental geochemistry and geochemistry in general, and thereby made his permanent mark on history.

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February, 1999

Keynote Lectures

THE ROLE OF RHIZOSPHERE PROCESSES IN THE BIOAVAILABILITY OF TRACE ELEMENTS TO PLANTS

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1. Introduction.

Because of the reactive nature of most of the essential micronutient metals in the soil, they are often found at vanishingly low ionic activities in the soil solution. Therefore, terrestrial plants have evolved a complex and elegant suite of strategies aimed at increasing the bioavailability of essential trace elements in the rhizosphere and transporting these metals into the root, in order to ensure adequate micronutrient nutrition for the plant (Marschner, 1995). Also, because many of the essential micronutrients have the potential to be phytotoxic (such as Fe), can be classified as toxic heavy metals (such as Zn, Cu, Ni), or are heavy metal analogues of micronutrients (Cd, Pb), their uptake has to be highly regulated. This talk will review the basic mechanisms and regulation of plant-based processes involved in increasing the solubility of trace elements in the rhizosphere and their subsequent absorption into roots.

For the portion of the talk dealing with essential trace element acquisition by plants, attention will be directed to the physiology, molecular biology and biochemistry of Fe and Zn acquisition. Because of the obvious linkages between essential trace element and toxic heavy metal acquisition by plants, the overlap between essential micronutrient and toxic metal mineral nutrition will also be discussed. This will involve a look at the mechanisms employed by several trace element hyperaccumulating plant species that have evolved on metalliferous soils and can accumulate trace elements to exceedingly high concentrations in the plant. Additionally, plant-based mechanisms for tolerating toxic levels of metals in the soil will be considered, which include avoidance (exclusion of the metal from the root) and sequestration of the metal in root vacuoles.

2. Rhizosphere Processes Involved in Fe and Zn Acquisition

Of all of the essential trace elements, plant Fe acquisition is the certainly the most widely studied and best understood (Kochian, 1991). Our current understanding of the different strategies used by specific plant species to enhance the bioavailability of Fe in the rhizosphere for subsequent root absorption will be discussed. These include the reductive-based strategy employed by dicot and non-grass monocot species, and the phytosiderophore-based strategy employed by grasses. The wide range of possible root-based modifications of the rhizosphere during Fe acquisition will be considered, including enhanced H^+ efflux, induction of the root ferric reductase system and Fe^{2+} membrane transporters, release of relatively non-specific low molecular weight organic ligands such as organic acids or more specific phytosiderophores, and changes in root architecture and structure (Briat and Lobreaux, 1997). For Zn acquisition, recent molecular research identifying plant Zn transport genes and their regulation by plant Zn status will be addressed (Grotz et al, 1998), as well as the role of root exudates in Zn bioavailability. There is some apparent overlap between Fe and Zn nutrition with regards to regulation of the acquisition of these two trace elements by plant Fe and Zn status that will also be covered. Finally, the role of the soil microflora in Fe and Zn bioavailability will be discussed, with a focus on soil bacteria for Fe acquisition, and mycorrhizae for Zn absorption from the soil.

3. How Plants Deal with Toxic Metals in the Soil

As the potentially toxic heavy metals in the soil are either essential micronutrients or micronutrient analogues, plant-based processes involved in solubilization and absorption of these metals in the soil must be highly regulated at the molecular and physiological levels. Thus the processes plants employ to obtain essential trace elements are also important for a variety of topics related to soil contamination with heavy metals. These include the entry of toxic heavy metals into the food chain (via uptake into plants), the phytoremediation of metal contaminated soils, and mechanisms of plant heavy metal tolerance.

One of the most fascinating aspects of plant responses to heavy metal stress involves the identification and recent physiological and molecular characterization of some of the heavy metal hyperaccumulator plant species. There are some 400 taxa of plants that have been identified that not only can tolerate and grow on soils contaminated with toxic levels of heavy metals, but also can accumulate these metals to exceedingly high levels in the plant shoot (1000 or more times normal shoot trace metal concentrations) (Baker and Brooks, 1989; Salt et al, 1998). Hyperaccumulators of the trace elements Ni, Cd and Zn have been identified and characterized. There is considerable interest in these hyperaccumulators both for their potential for phytoremediation, and also to better understand fundamental mechanisms of trace element accumulation and heavy metal tolerance. In this talk I will discuss mechanisms employed by Ni and Zn/Cd hyperaccumulators to acquire and accumulate these trace elelemtns to very high levels. Another aspect of trace element bioavailability that has been shown to be important to the emerging field of phytoremediation is the use of synthetic metal chelates to artificially induce hyperaccumulation of toxic metals from the soil into plant shoots. An example of this approach for Pb hyperaccumulation will be presented (Huang et al, 1997).

Finally, tolerance mechanisms that non-hyperaccumulator plants employ to deal with toxic levels of metals in the soil will be considered. There appear to be two main types of responses: avoidance and sequestration. The best characterized avoidance response is in cereal crop genotypes that exhibit tolerance to the toxic metal ion Al^{3+} in acid soils (Kochian, 1995). Although Al^{3+} is not a trace element, it will be instructive to consider the carefully regulated mechanisms that Al tolerant crop species appear to employ to exclude toxic Al from the root apex (the site of Al phytotoxicity). This strategy involves the Al-induced release of Al-chelating organic acids (citrate or malate) from the root tip into the rhizosphere, where Al^{3+} is chelated and detoxified. This response is localized only to the root apex, and may involve the Al gating of an anion channel that mediates organic acid anion release. The other metal tolerance response, sequestration in the root vacuole, will be discussed in relation to Zn tolerance and sensitive plant species.

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BIOAVAILABILITY OF TRACE METALS IN THE FOODCHAIN

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The principle pathways of trace elements in biological systems within the surface environment to be considered are those concerning soil - micro-organisms; soil – plant; soil – plant – animal; and soil – animal. The major inter-relationships affecting the dynamics between soil and plant have been discussed by Adriano (1986), Alloway (1995) and Allen (1997) and plant-based mechanisms are the subject of the parallel keynote address by Leon Kochian.

The complexity of the soil-plant-animal inter-relationships, including the importance of chemical forms and availability, interactions between trace elements, and between inorganic and organic substances were addressed in the early 1960's by Underwood (1962), and since by many scientists, including Mertz (1980) who reminded us that in the animal and human diet, "biological availability for absorption depends strongly on the form in which an element is supplied and this form varies from one dietary source to another", and that "biological availability and toxicity also depend on the concentrations of other trace elements present".

The term bioavailability is poorly defined and, in the present context, is considered to express either (a) that fraction of nutrient trace elements and/or potentially toxic heavy metals present in soils available for intake into plant roots or into soil micro-organisms and other flora and fauna or (b) that fraction in the soil, dust and/or diet that is bioaccessible (i.e. soluble and available for uptake in the gastro-intestinal tract of animals, including farm livestock, and humans.

Soils are the primary source of essential trace elements for plants and animals and therefore, for humans. Soils are also a source of heavy metals, though anthropogenic inputs of industrial and other emissions may greatly exceed natural geologic sources.

The availability of trace elements to micro-organisms, other soil fauna and plants is controlled by their total concentration in the soil and their chemical forms. Commonly referred to as speciation, these forms influence the partition of the metals between the solid and solution phases of soil; several chemical processes including absorption, precipitation and complexation are involved in this partition. Both total and soluble metal concentrations are also influenced by the processes of leaching, gleying, podzolization and surface organic accumulation, together with soil properties such as reaction (pH) and redox potential (Eh). The most important of these factors appears to be pH and the solubility and bioavailability of the majority of trace elements and heavy metals increase as pH decreases. Availability to sensitive receptors, such as micro-organisms and plants, will also be influenced by interactions with other major and trace elements present. For instance antagonistic reactions at root surfaces are found between the metal ions cadmium and zinc.

Generally, metals are taken up by plants via pore water. Metal ions non-specifically adsorbed to the solid phase may also be of importance as such ions are exchangeable with cations in solution and may readily enter solution. Free metal ion in water is usually considered to be the fraction

of the metal in soil available for interaction with micro-organisms and plant roots. However, as discussed by Allen (1997) it is more appropriate to consider that bioavailability is the consequence of competition for the metal between the organism, soluble ligands and sorbtion phases on the soil; thus the introduction of an invertebrate or plant to the soil-water system provides a competing receptor site for ions with soluble ligands and soil particles.

As with the pathway of trace elements from soil to plants, only a fraction of the total amount of most elements present in the diet will be absorbed and utilized for maintenance and production in the animal's body. Less is known about the forms in which trace elements are present in plants, though it is recognized that copper is mainly complexed with organic compounds of low molecular weight and with proteins (Kabata-Pendias and Pendias, 1984). It has been shown that an increase in concentration in the plant will not necessarily result in an increase in forms available to the animal; for example, copper in young grass has been shown to be less available to sheep than that in older herbage, even though the copper concentration was higher in the younger grass (Hartmans and Bosman, 1970). The availability of a trace element is frequently affected by the presence of interfering compounds, organic or inorganic in the diet. For example, dietary organic acids and amino acids may form chelates with elemental ions in the alimentary tract which will either enhance or inhibit the absorption of the element (Brebner, 1986)

For wildlife species and grazing livestock, the major source of essential elements is derived from ingested plant matter. Plants, in turn, depend on soil for their supply of mineral nutrients. The relation between trace elements in soils and plants and amounts absorbed and utilised by the grazing animal is again complex and depends on a variety of factors including the proportion of grass in the animal's diet, the digestibility of the diet, the form and availability of ingested trace elements and interactions with other dietary constituents. Several such interactions between dietary elements are recognized, including that between molybdenum, copper and sulphur, where high levels of molybdenum and sulphur in the diet of ruminants result in a decrease in copper absorption in the alimentary tract (Suttle, 1983). Low blood copper levels in impala in the Lake Nakuru National Park in Kenya were ascribed to both low soil and plant copper contents and high molybdenum content of some of the plants grazed (Maskall and Thornton, 1991). A further example of an interaction is shown by the influence of iron ingested in soil in inhibiting the utilization of dietary copper in calves (Humphries et al, 1985).

Animal factors also influence trace element uptake. For example, copper absorption and utilization has been shown to vary between breeds of sheep (e.g. Wiener and Field, 1970). The largest differences in absorption and utilization of dietary trace elements between species are those between ruminants and non-ruminants.

It was initially shown in the 1960's in New Zealand that animals can involuntarily ingest large quantities of soil while grazing (Healy, 1967). The majority of this soil is ingested accidentally along with herbage, as soil adhering to plant leaves following rain splash, trampling by animals or wind blow, as soil adhering to plant roots pulled up during grazing and as earthworm casts. The amount of soil ingested depends on the type of pasture and soil, stocking density, and climatic factors such as rainfall intensity. Typically, dairy cattle will ingest some 2 to 18 percent of their dry matter intake in the form of soil (Thornton and Abrahams, 1983). Sheep, which graze closer to the ground, may ingest greater quantities of soil, rising to 30-60 percent of dry matter intake. It has also been suggested that wildlife supplement their trace element intake through ingestion of soil at "salt licks" (Maskall and Thornton, 1991). The proportions of elements ingested as soil that are soluble, and thus bioaccessible, in the gastro-intestinal tract is

frequently low. For instance, in contaminated soils, the chemical weathering of metallic minerals in the soil environment over various timescales (recently termed ageing) may lead to the formation of minerals of extremely low solubility, such as pyromorphite (lead phosphate) and scorodite (ferric arsenate). The low solubility and limited bioavailability of lead-bearing minerals in mine wastes from Butte, Montana has been ascribed to the encapsulation of these in iron sulphide and silicate rinds (Davis et al 1994). Soil consumed may also have an important role to play in the nutrition of grazing livestock and has been shown in in-vivo experiments to supply cobalt to sheep where the ingested soil has enhanced rumen cobalamin production (Brebner, 1986).

Soil is subjected to a range of conditions within the rumen and alimentary tract of grazing animals which may affect the solubility and bioaccessibility of soil minerals. In this respect, while cobalamin production from ingested soil cobalt was rapid in sheep and was rapidly reflected in enhanced plasma cobalamin levels, extraction of cobalt in rumen was related to the size of soil particles ingested and soil type (Brebner, 1986).

Methods for determining the soluble and thus bioaccessible/bioavailable fractions of trace elements and metals in soils have been studied widely over the past 20 or more years. For the soil – plant/micro-organism pathway, these have included (a) the use of single chemical extractants of varying concentrations, such as EDTA, DTPA, acetic acid and 0.01 M $CaCl_2$, and (b) the use of operationally defined sequential extraction procedures, based on that employed by Tessier et al (1979) in which increasingly strong extractants are used to release trace elements associated with different soil fractions. The soil - animal pathway has been studied using (a) a simple method of simulating extraction within the stomach, using dilute HCl, (b) the application of a more sophisticated *in-vitro* technique – the Physiologically Based Bioaccessibility Test to similate the physiology of the stomach and the intestines, and using artificial enzymes and other digestive constituents as proposed by Ruby et al (1993). (c) a new perfusion method, based on continuous leaching of soil with 0.01 M $Ca(NO_3)_2$, developed by McLaughlin and co-workers at CSIRO (Australia) (personal communiction), (d) the use of the *in vitro* Rusitec artificial rumen system to similate extraction from dietary constituents and soils by cattle and sheep (McDonald, 1983) and (e) *in vivo* studies of absorption and utilization of trace elements in small animals (i.e. mice) and in fistulated livestock under experimental conditions.

Studies at Imperial College into the bioavailability of metals to sensitive receptor organisms, including micro-organisms and plants, comprises part of current research into critical loads of potentially toxic metals in the surface environment, and is described more fully in the paper by Rieuwerts et al to be presented later in this conference. Further current work, undertaken in collaboration with the University of Nottingham and the British Geological Survey, concerns metal speciation and bioavailability in brownfield sites in urban areas; integration of chemical extraction, scanning electron microscopy, and soil solution and vegetable analysis, with high precision isotopic analysis, will provide data in a GIS format for quantitative health-based risk assessment.

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Contents

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Phytoremediation
(Special Symposium 1)

THE GENUS *THLASPI* AS A SOURCE OF PLANTS FOR PHYTOREMEDIATION STUDIES

REEVES ROGER D. [1], BAKER ALAN J.M. [2] and DAVID LLOYD-THOMAS D. [3]

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1. Introduction

Work by A. Braun and F. Risse, reported by Forchhammer (1855) and Sachs (1865), respectively, showed the extraordinary zinc-accumulating properties of two plant species (then called *Viola calaminaria* and*Thlaspi alpestre* var. *calaminare*) of the metalliferous (lead-zinc) soils of the Aachen-Liège region (Germany-Belgium). The latter species, in particular, can contain more than 1% Zn in its dry matter, or 10% Zn in the ash. This observation was rediscovered several times during the next 120 years, and more extensive studies on Zn uptake by the *Thlaspi* species, in particular, both in Germany and the UK, were reported. The latest revision of *Thlaspi* in Europe has taken many of the variants previously grouped under *T. alpestre* and regards them as *T. caerulescens*, the first valid specific name, used by J. & C. Presl in describing a Czech population in 1819. Most recent workers have adopted this nomenclature.

Similar Zn-accumulating behaviour was reported by Rascio (1977) for *T. rotundifolium* ssp. *cepaeifolium* growing over Zn deposits near the border of Italy and Austria. The observation that many *Thlaspi* species occurred on serpentine soils led to the discovery of nickel hyperaccumulation by many of the 70 recognised *Thlaspi* taxa in Europe and North America (Reeves & Brooks, 1983; Reeves *et al.*, 1983), Japan and Turkey (Reeves, 1988). The common weedy annuals (e.g. *T. arvense, T. perfoliatum)* do not show remarkable metal uptake, even when they occur on metalliferous soils.

Studies on serpentine and non-serpentine populations of the Austrian *T. goesingense* (Reeves & Baker, 1984) showed this species to have an innate ('constitutive') ability to tolerate and accumulate metals from metalliferous soils of types to which their populations had never previously been exposed. The ability of British populations of *T. caerulescens* to accumulate a wide variety of elements, either in the roots or in the whole plant, from extremely dilute solutions, has also been reported (Baker *et al.*, 1994). Recent work has provided several further important observations relevant to the use of *Thlaspi* species in phytoremediation.

2. Materials and Methods

Many hundreds of *Thlaspi* specimens, both from Herbarium collections and from populations sampled directly from the field, have been subjected to multielement analysis by atomic absorption or ICP. Particular attention has been paid to Zn, Cd, Pb, Ni and Co. Collections made in the field have been accompanied by soil sampling and analysis.

3.Results and Discussion

Contrary to statements sometimes made, *T. caerulescens* is not an absolute metallophyte, but occurs in several countries (e.g. France, Scotland, Spain, Sweden) on a variety of non-

metalliferous soils (limestone, granite, shale, quartzite). Even from these soils, with only 50-400 mg/kg Zn, very high plant Zn (3000-20,000 mg/kg) can be observed. These observations parallel those recently made (Meerts and van Isacker, 1997) on populations in Luxembourg. Some populations of *T. caerulescens* and *T. ochroleucum* on zinc-rich soils show high Cd concentrations, and Ni levels that are high for plants on non-serpentinitic soils can also be encountered.

The very rare Turkish species *T. cariense*, discovered in 1983, and still known from only two locations, is a hyperaccumulator of Ni (up to 18700 mg/kg in senescent leaves and 12000 mg/kg in rosette leaves and siliculae). This, and other larger Turkish Ni hyperaccumulators, such as *T. elegans, T. rosulare, T. oxyceras* and *T. jaubertii*, growing up to 60 cm, may be of more value for phytoremediation than *T. caerulescens.* Better knowledge of their distribution, rarity, life cycle and behaviour towards a greater variety of metallic elements in soil or in solution, is still needed.

In the USA, all taxa under *T. montanum* are hyperaccumulators of Ni when growing on serpentine, but also show considerable elevation of Zn from substrata with normal Zn concentrations. European specimens of *T. montanum* on serpentine have shown only slight elevation of Ni, and no abnormal Zn.

4. Conclusions

(1) Many populations of *T. caerulescens* accumulate Zn to very high concentrations even though they are not on Zn-rich substrata. (2) Several (perhaps most) of the biennial or perennial species (including *T. caerulescens, T. goesingense, T. ochroleucum* and American populations of *T. montanum*) are capable of multielement hyperaccumulation, with a preference for Zn and Ni, but some species have been shown to be capable of accumulating Cd, Co and Mn to high concentrations throughout the plant, and Cu, Pb, Cr and Fe to high concentrations in the root. (3) The serpentine-endemic *T. cariense* is a Ni hyperaccumulator. (4) Several species, e.g. *T. elegans, T. jaubertii* and *T. cariense*, may be better producers of biomass than *T. caerulescens*, and may hold more promise for remediation; more work on these is needed. (5) The sporadic distribution of all the metal-accumulating *Thlaspi* species raises important questions regarding their conservation.

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PHYTOEXTRACTION OF METALS FROM CONTAMINATED SOILS

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1. Introduction

Human activities lead to the ineluctable increase of metal concentration in soils. Becoming a threat for the functioning of the surrounding ecosystems and for food safety, contaminated soils should be treated to extract the metals or reduce their mobility. An alternative to physical and chemical methods is the use of hyperaccumulator plants which may extract the excess metals from polluted soil, i.e. phytoextraction (Baker *et al.*, 1994; Brown *et al.*, 1994). Hyperaccumulators are a special class of plants which have acquire the ability to accumulate concentrations of metals higher than 1% in the foliar dry matter. Hyperaccumulators have been tested in various laboratories around the world since the beginning of this decade to test their behaviour on contaminated soils, and decrease the amount of metal in soil.
This paper relates experiments conducted with two hyperaccumulator plants, *Thlaspi caerulescens* and *Alyssum murale*, to assess their ability to grow on polluted soils, develop their root system, respond to agricultural practices as fertilisation, and to meet clean-up goals.

2. Growth of hyperaccumulator plants on polluted soils

Seeds of *T. caerulescens* and *A. murale* were collected from former smelter sites in Belgium, France and Albania. Plants were grown on various materials, i.e. agricultural soil, zinc-smelter site, nickel smelter site, lead factory, and dredged sediments (Schwartz, 1997). Both were able to grow on these polluted materials. In presence of zinc, growth of *T. caerulescens* was increased, and higher metal concentrations in leaves were observed on the most polluted soils, and at lower pH. On soils low in Zn but high in Pb or in Cd, plants showed an accumulation of Pb or Cd.

3. Root system of *T. caerulescens* according to localisation of pollution in soil

The use of rhizotrons showed the preferential development of the root system of *T. caerulescens* in areas where metal (Zn or Cd) was present at high concentration in soil (Schwartz *et al.*, 1997; Perronnet, 1997; Saison, 1998). Also, roots exhibited morphological changes in the most polluted zones as well as modifications of soil pH in their rhizosphere.

4. Response of *T. caerulescens* and *A. murale* to N and P fertilisation

Increasing rates of phosphorus and nitrogen (ammonium or nitrate) were applied to polluted soils and the response of *T. caerulescens* and *A. murale* to fertilization were recorded. Both plants responded to fertilization by a higher biomass production than in control. Increase in biomass was higher with nitrogen and depended on the form of the nitrogen supplied. A slight decrease in metal concentration was recorded, but it was lower than that predicted from the simple dilution effect. Therefore, phytoextraction of Zn and Cd by *T. caerulescens,* and of Ni by *A. murale* was augmented by mineral fertilization.

5. Contribution of hyperaccumulator plants to meet clean-up criteria

<u>Total content of metals in soils</u> : phytoextraction is aimed at decreasing the danger linked to polluted soils. Efficiency of this emerging technology is generally based on the total content of toxic trace elements in soil. A field experiment was conducted to study the extraction of Zn and

Cd by *T. caerulescens* from an agricultural soil polluted with a metal rich urban sludge (24 mg Cd kg^{-1} DM and 2 347 mg Zn kg^{-1} DM) (Schwartz, 1997). At harvest, shoot dry biomass and leaf tissue metal content were measured for each plant. Extraction of metals was increased by the increase in biomass, and the largest plants (> 5 g DM plant) contributed more to Zn extraction than small plants (< 1 g), despite a slight reduction in the metal concentration in plant tissues of larger plants. Growth of *T. caerulescens* significantly decreased the total metal content in soil.

Phytoextraction depletes the pools of available metals in soils : since the danger is directly related to the mobility and availability of the elements, priority clean-up goal should be the decrease of the concentration of the labile forms. Normal plants generally take up their elements from the most mobile pools, i.e. phytoavailable pools. Using isotopic methods, we have demonstrated that the hyperaccumulator plants take up their metals, as any cultivated plant, from the pool of isotopically exchangeable metal ions which represent the actual phytoavailable metal (Shallari, 1997; Gérard, 1997). Therefore, hyperaccumulators have a particular efficacy to deplete the available pool of metals, and, in general, water-, ammonium nitrate- or DTPA-extractable fractions of metals are reduced after harvest.

6. Conclusion

Phytoextraction is the use of hyperaccumulating plants to remove toxic metals from polluted soils. An efficient technology requires the production of high biomass with high concentration of metals in the tissues. The potential of hyperaccumulator plants to extract significant amounts of metals from polluted soils, and deplete the compartments of the most mobile metal ions has been demonstrated. Plant species responded well to mineral fertilization which enhanced biomass production without reduction of the metal uptake. Our results suggested that selection should focus on hyperaccumulator plants instead of plants which have only a slight ability to accumulate but produce high amounts of biomass.

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CRITICAL FACTORS FOR THE APPLICATION OF PHYTOREMEDIATION IN METAL CONTAMINATED SOILS

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1. Introduction

The use of soil amendments to increase the availability for metal uptake by plants has raised some concern over the potential increased mobility of the metal-chelate complex in the soil. Because lead and other heavy metals in the soil are relatively immobile, insoluble and unavailable for plant uptake, the addition of chelating agents and other soil amendments are necessary to increase the plant availability and uptake of the metals as part of any phytoremediation activity. However, through the proper use and selection of soil amendments, phytoremediation can remove heavy metals from the soil through plant uptake with minimal risk to the groundwater.

Soil amendments used for phytoremediation of metal contaminated soils usually contain synthetic or naturally occurring chelating agents. Two of the most common chelators used are EDTA (ethylenediaminetetraacetic acid) and citric acid. The chelating agents are applied to the soil surface through the irrigation system at specified stages of plant growth. The first effect of the chelator is to increase the solubility of the metal in the soil through the formation of a chelate-metal complex. The chelate metal complex is then absorbed by the plant root where the chelate enhances transport of the metal complex to the shoots of the plant. The plant biomass can then be harvested and removed from the site resulting in a decrease in the soil contaminant.

The increase in metal solubility as a result of the addition of phytoremediants to the soil creates a potential risk of leaching or downward migration of the metal. The potential risk involved with the application of chelating or solubilizing agents becomes a factor of the length of time the chelate remains active in the soil and the mobility of the chelate metal complex. The metal chelate complex is subject to several fates: 1) plant uptake of the metal chelate complex, 2) sorption of the chelate or metal-chelate complex by the soil components, 3) biological degradation or deactivation of the chelate, and 4) leaching of the chelate to a lower depth in the soil profile.

Plant uptake removes a significant amount of the applied chelate from the soil. Recent work by Vassil et al. (1998) and Epstein et al. (1998) in greenhouse studies have shown shoot dry weight concentrations of EDTA to be as high as 46,000 mg/kg, accounting for as much as 10% of the applied chelate. While not all of the chelate is removed from the soil, the most mobile forms will be accumulated by the plant tissue.

Recently Phytotech has conducted several studies to evaluate the movement of EDTA and associated soluble metals in soils as a result of the application of phytoremediants and the phytoremediation process.

2. Materials and Methods

Soil columns were constructed using 18" long, 3" diameter PVC pipe sections. The column was fitted on one end with a Buchner funnel and sealed with silicone and placed in vertically in a stand. A 7.5 cm diameter Whatman No. 42 filter paper disk was placed in the bottom of each column and the columns were filled with sieved soil (2.27 kg) to within 1 inch of the top of the column. The soil in each column was saturated with water by adding to the soil surface until water drained from the bottom of the column. The surface of the column was covered and the column was allowed to drain for 48 hours to remove gravitational water. EDTA was added to the soil surface using a 1.1 M solution at the rate of 0.075 mL/cm^2. Water was applied to the soil column using volumes of 0.25, 0.5, 1.0, 2.5 or 5.0 inches (29, 58, 115,232, or 579 mL, respectively) using duplicate treatments for each volume. Columns receiving treatments of 2.5 or 5.0 inches of water produced solution from the bottom of the column.

The surface of the columns were covered with plastic film and the columns allowed to stand for seven days. The columns were then cut open and soil samples collected at three-inch depth intervals. The soil samples were analyzed for water soluble metals and EDTA in a 1:10 soil:water suspension.

3.Results and Discussion

The application of 5 inches of water resulted in the deepest movement of EDTA and a corresponding increase of soluble lead to a depth of 9 inches. The highest levels of water soluble lead were restricted to the top 0 to 3 inches of soil except those columns receiving 5 inches of water where the highest soluble lead levels were found at the 3 to 6 inch depth. No soluble EDTA was detected below the nine inch depth in any of the soil columns. EDTA was also not detected in the leachate from any of the columns.

Phytotech has measured similar results in field phytoremediation trials where no correlation between surface soil lead concentration and subsurface water soluble lead concentrations was observed indicating that the phytoremediation activities did not incrrease the movement of surface lead or chelate to the subsurface soil.

Sorption and degradation of EDTA can remove a significant portion of the applied EDTA from the soluble pool. Plant uptake of the metal chelate complex will also result in additional decreases in the soil solution levels. Data collected in laboratory, greenhouse and field studies have not shown any increased movement of lead or chelate into the subsurface soil as a result of soil amendments. When the existing groundwater is several meters or more below the soil surface, the risk of groundwater contamination by the metal or chelate due to phytoremediation is minimal. For those sites where a shallower water table exists, additional characterization work may be necessary to evaluate the use of soil amendments as part of the remediation plan.

PHYTOREMEDIATION, FIELD TRIALS IN THE YEARS 1993-1998

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1. Introduction

In the present publication the current state of the art of phytoremediation of soils polluted by heavy metals will be shown as well as needed improvements. Results of field trials from the years 1993 to 1998 will be presented. Special emphasis is put on mobilization of heavy metals in soil to improve the transfer of metals from soil into plants.

2. Materials and Methods

Plants, trial sites and the experimental design have been described (Felix, 1997).

3. Results and Discussion

The ability of crops and wild species to accumulate cadmium in aerial parts looks as follows:

Plants	harvest yield (t/ha)	Cd-content in plants (mg/kg dw)
Alyssum murale	0.8	34
Thaspi caerulescens	16	12
Nicotiana tabacum	9	10
Zea mays (B37 inbred line)	10	8
Brassica juncea	21	3
Salix viminalis	10	22

A. murale mentioned to be a good accumulator of nickel was the best cadmium accumulator in the present study. Remarkable results were also obtained with the tree *S. viminalis*.

Assuming a linear removal of Cd from the field *S. viminalis* would need 77 years to decrease cadmium concentration from 6.6 mg (concentration in the field) to 0.8 mg/kg (target value in Switzerland). Crop plants like corn and tobacco known to accumulate Cd are not as efficient as *S. viminalis*. *B. juncea* described to be generally a good metal accumulator did not perform too well in this study.

The next table shows the ability of two wild species to accumulate zinc in shoots:

Plants	harvest yield (t/ha)	Zn-content in plants (mg/kg dry weight)
Alyssum murale	0.6	675
Thaspi caerulescens	13	2545

From literature *T. caerulescens* is known to be an accumulator for zinc.

Taking yields into account and assuming a linear removal, *T. caerulescens* would need 54 years to decrease zinc concentration from 810 mg to 200 mg/kg (target value in Switzerland).

The last table shows the ability of *B. juncea* to accumulate lead in shoots:

Experiment	Pb soluble in soil (g/ha)	Pb-content in *B. juncea* (mg/kg dw)
control	0.002	0.1
NTA added	0.02	0.2
EDTA added	0.2	0.6
NTA + glyphosate added	0.03	0.3
EDTA + glyphosate added	0.2	1.7

For this study a heavily contaminated site was chosen (up to 10 g Pb/kg). From literature *B. juncea* is known to accumulate considerable amounts of Pb (Huang et al., 1997; Blaylock et al.,

1997), in particular when chelating agents are applied. Compared to the control the addition of the chelating agents NTA and EDTA increased the soluble (plant-available) lead content in the soil by a factor of 9 or up to 100 respectively. As a consequence also the Pb content in plants was more than five times higher after EDTA treatment. Treatment of the *B. juncea* plants with glyphosate eleven days before harvest increased Pb up-take further: The combination of EDTA and herbicide treatment resulted in a 15 times higher Pb concentration in plants compared to the control, but clean-up even of moderately contaminated site would still need a long time.
The results demonstrate that several plants have an unusual ability to accumulate Cd, Pb and/or Zn from solid substrates. *A. murale*, *T. caerulescens* and *B. juncea* as well as the tree *S. viminalis* have the highest metal-accumulating ability among the species tested (not all data were shown).
According to literature data *Thlaspi* species could take up much higher amounts of Zn and Cd (Ernst, 1975): 25'000 mg Zn and 560 mg Cd per kg dry matter are mentioned compared with 2545 and 12 mg/kg found in this study: A ten- or fifty-fold increase seems possible for Zn or Cd, respectively. Literature data (Collins, 1981; Jørgensen, 1993) indicate that an increased amount of bioavailable metals in soil solution gives rise to an increased uptake of heavy metals into plants. If the plants are watered with EDTA solutions, it is possible to dissolve a significant fraction of the heavy metals and a high concentration of heavy metals in the plants is achieved (Jørgensen, 1993). The release of organic exudates by the plant modifies the flow and availability of plant nutrients as well as toxic metals such as Cd by acidification, chelation and precipitation reactions, whereby the soil rhizosphere in relation to Cd speciation and availability plays an important role (Krishnamurti et al., 1996). In recent publications (Huang et al., 1997; Blaylock et al., 1997) the addition of complexing agents was proposed to increase metal up-take. Accordingly complexing agents in equimolar amounts to the metal contents in soil were added. The application of complexing agents in the Pb trial in fact had dramatic effects on the soluble content of Pb in the soil as well as the up-take of Pb by the plants. There is, however, at least one severe drawback: Solubilized Pb could enter the groundwater.
The effect of the herbicide glyphosate is not easy to explain: It can be imagined that plants treated with e.g. glyphosate are stressed and take up more water together with solubilized lead.
The use of plants to clean up soils contaminated with heavy metals only makes sense if the period needed is below about five years. None of the plants fulfils this demand under present experimental conditions: Either yields or metal up-take rates are still too low.

4. Conclusions

Research has to concentrate on the investigation of further plant varieties (somaclonal variation, genetic engineering), physiological needs of hyperaccumulators (temperature, soil, humidity, fertilizer, soil treatment), and in particular mobilization of metals using complexing agents, fertilizers and plant exudates.

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CONSTAINTS TO THE GROWTH AND METAL UPATKE BY HYPERACCUMULATOR PLANTS

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1. Introduction

Hyperaccumulator species, or future plants that have the traits for hyperaccumulation and hypertolerance transferred into them by either conventional or genetic techniques, could be of great value for extracting metals from land. They have applications either for environmental clean up or biomining of metals. The effects that the plants have on their environment, or conversely, the effects that environment or soil treatment have on the growth and metal accumulation and transport within the plant must be understood, as these may affect both the efficacy and applicability of phytoextraction or phytomining at different sites.

2. Materials and Methods

The Zn/Cd/Ni hyperaccumulator species *Thlaspi caerulescens* was grown in solution culture, soil or commercial potting compost, as appropriate to each experiment (see references).

3. Results and Discussion

T. caerulescens sown on acidified soil did not germinate at pH 4, and did not grow well on soils of pH <5. Therefore, methods that increase the metal extraction efficiency by acidification of the soil or substrate can only be used within this constraint.

In both hydroponic and soil experiments, high Zn uptake by *T. caerulescens* was not correlated with acidification of the rhizosphere (McGrath et al, 1997, Knight et al, 1997). This confirms work with the Ni hyperaccumulator *Alyssum murale* which also did not acidify the rhizosphere (Bernal and McGrath, 1994, Bernal et al, 1994). Coupled with this lack of acidification, *T. caerulescens* actually decreased the Zn concentration in soil solution by amounts that varied in relation to the soil type. On average, 99% of the total Zn uptake by plants was derived from the solid phase, through the soil solution (Knight et al, 1997).

T. caerulescens appears to have a high Zn requirement. In solution culture, the total dry weight increased significantly when solution concentration increased from 1 to 10 µmol. For normal plants, 1 µmol is sufficient Zn supply (Shen et al, 1997). Using chelate buffers in hydroponic systems with controlled Zn activities, *T. caerulescens* had an external Zn requirement approximately 5 orders higher than most plant species (McGrath, 1998). Shoot yield increased by 75% when 1000 mg Zn kg^{-1} was added to a commercial compost containing 88 ppm Zn (Küpper et al, 1998).

Because *T. caerulescens* takes up so much Zn, this may interfere with its P nutrition, due to the formation of insoluble Zn phosphates inside the plants. However, it has now been shown that *T. caerulescens* can efficiently separate the P and Zn inside the plant in different leaf cells. Much of the accumulated Zn is present in the vacuoles of the epidermis, whereas more P is in the mesophyll cells (Küepper et al, 1998). Even with very high Zn supply (500 µmol Zn), there was no evidence of P deficiency in the plants. Also, foliar applied P did not improve plant growth (Zhao et al, 1998).

Although *T. caerulescens* is very tolerant of Zn, Cd and Ni, it may be sensitive to high Cu concentrations (Schat et al, 1997), and may therefore not grow in Cu-contaminated soils.

Soil amendments have been used to increase uptake of metals by non-hyperaccumulator plants. This was tested on *T. caerulescens* in the field in 1998, by adding the organic complexing agents citric acid, NTA or EDTA to the soil. None of these increased Zn or Cd concentrations, but EDTA increased Cu markedly and Al uptake slightly. Additionally, EDTA inhibited the growth and survival of the plants, possibly due to Cu, Al or EDTA toxicity.

4. Conclusions

Zn hyperaccumulator plants such as *T. caerulescens* may not grow in acid soil, in soil low in available Zn, or soils high in copper. On the other hand, high phosphate does not seem to inhibit either the P or Zn uptake, so this is not a limitation. The fact that they become zinc deficient in soils with 'background' zinc concentrations is seen as a useful trait. This means that the plants a) are not likely to escape from polluted sites and b) will stop growing well when the highly available Zn has been extracted.

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ECOLOGICAL CONSEQUENCES OF METAL ACCUMULATION AND HYPERACCUMULATION BY PLANTS

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1. Introduction

The responses of plants to high edaphic metal concentrations include sensitivity, populational tolerance, and constitutive tolerance. Tolerant plants have been categorized in their metal uptake properties as excluders, indicators, accumulators, or hyperaccumulators (Baker & Walker, 1990). Most cases of metal tolerance involve evolution of locally tolerant populations (Antonovics et al. 1971). Although constitutive tolerance is much less common, it is among these plants that most examples of accumulation and hyperaccumulation may be found. Recent interest in accumulation and hyperaccumulation as resources for phytoremediation of contaminated soils necessitates an understanding of the ecological and evolutionary consequences of these processes in nature.

Several adaptive/evolutionary hypotheses have been proposed to explain the rare phenomenon of hyperaccumulation. Although hyperaccumulation and tolerance are clearly correlated, the causal connection between the two is obscure. It has been argued that hyperaccumulation may directly confer tolerance or, conversely, that tolerance permits hyperaccumulation to evolve. We will evaluate these hypotheses, along with recent evidence that hyperaccumulation may confer selective advantages by defending plants against damage by herbivores or pathogens. Less is known about the ecology of metal accumulation below the thresholds considered to represent hyperaccumulation. We have recently carried out experiments to determine whether moderately elevated zinc concentrations in *Thlaspi caerulescens* deter feeding by herbivores. These studies were designed to test whether herbivore selectivity is expressed in a heterogeneous population of plants with differing metal concentrations across the range of accumulation and hyperaccumulation.

2. Materials and Methods

Seeds from a British population of *Thlaspi caerulescens* (Brassicaceae) were germinated and grown hydroponically in five nutrient solutions with differing zinc concentrations: 0.01, 0.10, 1.0, 10.0, and 20.0 mg L^{-1} Zn (as $ZnSO_4$). Sample leaves were harvested, dried, weighed, ashed, dissolved in nitric acid, and measured for zinc content using flame atomic absorption spectrophotometry. Plants were transferred to randomized feeding arrays consisting of ten plants (two from each treatment group). Two larvae of *Pieris rapae* (Lepidoptera: Pieridae) were allowed to feed within each array for three days. Number of leaves per plant was scored before and after feeding. The relationships between solution concentration, foliar concentration, and herbivore damage were explored through correlation analyses.

3. Results and Discussion

Foliar zinc concentrations ranged from 400 to 50,000 μg g^{-1}. Mean foliar concentrations were strongly correlated with growth-solution concentrations ($r = 0.997$); however, the two lowest solution concentrations generated plants with almost identical foliar [Zn]. These treatments have

therefore been combined in the analyses that follow. There was a highly linear negative relationship between foliar zinc concentration and mean number of leaves eaten (r = -0.998; Fig. 1), suggesting that herbivores prefer plants with lower metal concentrations.

Previous studies of herbivore responses to metal accumulation (e.g. Martens & Boyd 1994; Pollard & Baker 1997) have utilized artificial contrasts between plants of extremely high and extremely low metal concentrations. Genetic studies, on the other hand, (Pollard & Baker 1996) suggest that variation in natural populations is continuous, without clear-cut polymorphisms between hyperaccumulating and non-accumulating plants. Our experimental design was intended to simulate more closely the foraging behavior of herbivores in a variable population. Our finding that the negative relationship between feeding and zinc concentration extends to plants below the 10,000 ppm criterion for hyperaccumulation suggests that herbivory might select for the evolution of hyperaccumulation from an ancestral population of plants with more modest powers of metal accumulation. We are continuing to examine this question, and will present the results of these ongoing studies at the conference.

4. Conclusions

Hyperaccumulation of heavy metals deters feeding by herbivores, and lower levels of accumulation may also affect herbivore preferences. Accumulation of metals in plant tissues represents a potential avenue for movement of these elements into animal food chains. Studies of the rates at which such ecosystem fluxes occur must consider both the concentration of the trace element in the plant, and the acceptability of that plant to herbivores. It is possible that accumulators represent a greater source of metals at the ecosystem level than do hyperaccumulators, because of the reduced acceptability of hyperaccumulators to herbivores.

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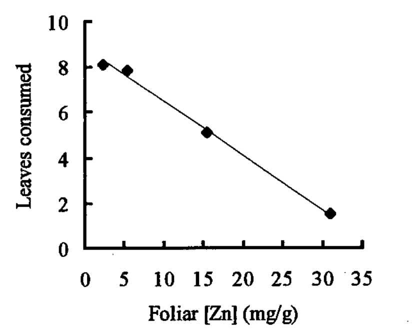

Fig. 1: Effect of zinc in *Thlaspi caerulescens* on feeding by larvae of *Pieris rapae.*

PHYTOEXTRACTION OF SOIL NICKEL USING *ALYSSUM* SPECIES

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1. Introduction

Phytoextraction is a promising soil remediation technology being developed for several trace elements. Metal contaminated soils comprise an environmental hazard in many locations. Where soil remediation is required, such soils also represent an opportunity for using hyperaccumulator plant species to phytoextract soil metals for reduced remediation cost or for profit. It appears that if improved cultivars of natural wild hyperaccumulator plants can be grown and harvested like hay, the biomass burned or pyrolized for energy value and ash recovery, and metals recovered from the ash for marketing, an economic technology will result. For Ni, selected plants can accumulate over 400 kg/ha, with low crop production costs similar to wheat. To build such a phytomining technology, one must collect and evaluate wild germplasm, breed improved genotypes, and identify fertilization requirements and agronomic practices for optimum production (Chaney et al., 1999). In 1998, Viridian Environmental joined USDA-ARS, the University of Maryland, and the Environmental Consultancy of the University of Sheffield in a Cooperative Research and Development Agreement to develop and commercialize a complete phytomining and metal recovery industry; Oregon State University is cooperating in field testing.

2. Materials and Methods

We collected genetically diversified germplasm of *Alyssum* species which can hyperaccumulate shoot Ni to >1% Ni from serpentine soils. Greenhouse pot tests and field tests were conducted using serpentine or Ni-refinery contaminated soils from several locations. We used 4 L pots, and supplied needed fertilizers at rates to which plant yield or metal hyperaccumulation were expected to respond. Reagent grade nutrient sources were used. For acidification, dilute nitric acid was added according to pre-tests to attain set pH levels, incubated 4 days, and the soil leached with deionized water to remove dissolved Mg nitrate. Strains of *A. murale* and *A. corsicum* were grown for 60 or 120 days before harvest of whole shoots. For selected tests, leaves were separated from stems and petioles for analysis. In the field, 6 m long rows spaced 50 cm apart were sown using V-belt seeders to test genotypic differences in yield and Ni hyperaccumulation. In May, 1998, single branch stems were collected from 5 plants per row so that analysis could support selection of parents for plant breeding, and at flowering or later August, whole shoots were harvested for analysis. Plants were dried, ground, dry-ashed, and analyzed by ICP-OES with Y internal standard. Soil total metals were extracted by hot nitric acid, and neutral salt extractable metals by 0.01 M $Sr(NO_3)_2$.

3. Results and Discussion

Species studied are native to serpentine soils which are very deficient in Ca and P for crop plants,and which fix added P strongly. We found that Ca did not inhibit Ni hyperaccumulation, and increased tolerance of plant shoots to Ni. Further, although P was the most limiting fertilizer

nutrient needed to maximize biomass yield on serpentine soils, exceptionally effective P uptake and utilization by these species kept required P applications low. Ca and other elements were absorbed to adequate levels, but some elements would have to be replaced to maintain soil fertility. Field planting methods were developed. Herbicides for practical production were also identified. Perennial culture appears to give some advantage economically. Crop planting and harvesting methods must be adapted to local climates. Leaves had approximately double the Ni level as stems so harvest before leaf drop during seed filling is important in retaining Ni in harvested biomass. Plants grown with appropriate fertilizers and production practices, harvested at one year after seeding, contained 8000-20000 mg Ni/kg on a colluvial soil with only 660 mg total Ni/kg. Results of the genetic evaluation indicate that sampling and selection can be conducted in one crop year to aid in efficiency of breeding. Genetic variation appears adequate to support breeding to improve both Ni levels in shoots and shoot yields. Field tests were also conducted on smelter contaminated soils from Canada with effective hyperaccumulation. Figure 1 shows the relationship of Ni concentration in branch stems in May compared with whole shoots at harvest for genotypes harvested on both dates.

4. Conclusions

At the 1997 ICOBTE meeting, we reported our view of improving hyperaccumulator species to develop effective phytoextraction technologies, and that one must learn how to grow high yields with high metals levels using conventional agronomic practices (Chaney et al., 1999). A utility patent has been awarded for our overall technology (Chaney et al., 1998) and Plant Variety Protection Certificates applications submitted for improved genotypes. We conclude that development of effective agronomic management practices and improved cultivars of hyperaccumulator species shows promise for development of successful phytomining and phytoremediation technologies for Ni. Success with development of hyperaccumulator plants for phytoextraction of other elements will depend on the properties of plant species available.

Fig. 1. Correlation of Branch-shoot Ni with Whole Shoot Biomass Ni concentration at Harvest for *Alyssum* genotypes. Fig. 1. Correlation of Branch-shoot Ni with Whole Shoot Biomass Ni concentration at Harvest for *Alyssum* genotypes.

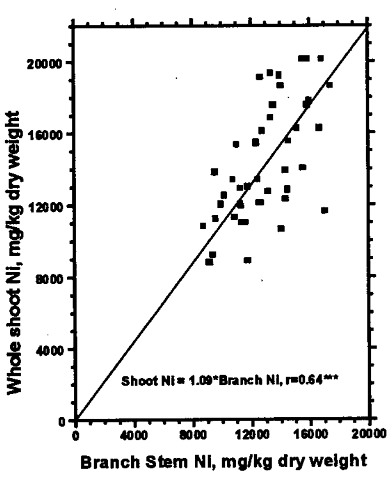

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Fig. 1. Correlation of Branch-shoot Ni with whole Shoot Biomass Ni concentration at Harvest for *Alyssum* genotypes.

BENEFITS, LIMITS AND EVALUATION OF LONG-TERM EFFICACY OF PHYTOSTABILIZATION AND *IN SITU* INACTIVATION OF METAL CONTAMINATED SOILS

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Phytostabilization whether or not combined with *in situ* inactivation (immobilization) of metal contaminated soils is a developing technique which seems to be a valuable alternative for more expensive and complicated civil-engineering techniques. The use of soil amendments and plants to alter the chemical and physical form of the heavy metal contaminants in the environment thereby decreasing the chemical and biological ability to cause harm was shown to be very promising (for a review see Vangronsveld and Cunningham 1998).

Plants perform two principal functions in phytorestoration by protecting the contaminated soil from wind and water erosion, and reducing water percolation through the soil to prevent leaching of contaminants. Plants may also help to stabilize contaminants by accumulating and precipitating heavy metals in the roots, or by adsorption on root surfaces. Less commonly, plants may assist in altering the chemical form of the contaminants by changing the soil environment (e.g., pH, redox potential) around plant roots. The micro-organisms (bacteria and fungi) living in the rhizosphere of these plants also may have an important role in these processes.

The main objective of phytostabilization and *in situ* immobilization is to decrease the risks of the contamination for the environment and human health through reductions of relative mobility and bioavailability of the metals in the soil. A more thorough evaluation of the overall effect of ameliorants and the sustainability (durability) of metal immobilization in contaminated soils may combine physico-chemical and biological methods. Metal speciation work with x-ray diffraction or x-ray absorption techniques can be used to support selective or sequential extraction methods, providing valuable data on the forms of metal in the soil. An understanding of the forms of contaminants present in the soil can be used to make reliable predications about sustainability of the *in situ* inactivation.

Biological methods complement physico-chemical evaluation methods, which do not directly address biological availability or toxicity. Several case studies demonstrated that amendments decreased the soluble and exchangeable metal fractions, but that changes in metal uptake by plant were not significant. Not only toxicity but also possible soil additive induced deficiencies of essential elements must be considered.

Biological evaluation should be performed using various living organisms from different trophic levels; existing ecotoxicity tests can be used, but new ones may need to be developed. Some microbial assays can detect specific categories of toxicants such as trace metals.

Another important parameter to monitor is the evolution of biodiversity. Increase of biodiversity (plant species, mycorrhiza, soil bacteria, invertebrates, ...) is a good indicator for the quality of immobilization. The establishment of f.i. a mycorrhizal network in revegetated areas is thought to be essential for the development of a sustainable ecosystem, while highly mycotrophic plants are characteristic of stable, sustainable ecosystems.

Other aspects of monitoring may include *in situ* plant sampling to determine plant tissue concentrations of metal contaminants. Plant sampling may indicate whether or not treatment is effective, and if a hazard exists due to human or animal ingestion of plants on the site. Along with contaminant monitoring, soil sampling for fertility purposes should be conducted on a regular basis (every 3 to 5 years) to indicate fertilizer and other requirements.
In this presentation the combined use of sequential extraction procedures, microbial metal biosensors, phytotoxicity tests and a zootoxicity test is presented as a test system for evaluation and monitoring of efficacy and durability of *in situ* inactivation of metal contaminated soils.
The importance of the combined use of physico-chemical and biological methods for monitoring the evolution of immobilization will be illustrated by several examples. Combined studies of microbial biosensors, bacterial ecology, phytotoxicity and zootoxicity, were tested in a comparative study of the efficacy and durability of several soil additives on a gradient of increasing metal concentration in Zn, Cd, Pb and Cu contaminated soil. Physico-chemical evaluations were complemented with these data. Rather good correlations were found between different parameters. The correlation between exchangeable (= $Ca(NO_3)_2$ extractable) zinc is given as an example in Figure 1.

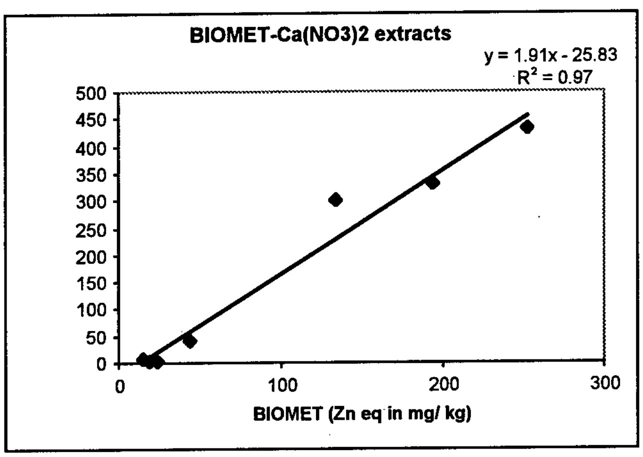

Figure 1: Comparison of bioavailable Zn determined with the BIOMET AE1433 test (in Zn equivalents per kg) and exchangeable (= $Ca(NO_3)_2$ extractable) zinc in a heavily polluted soil from an old zinc smelter site treated with different soil additives.

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ACCUMULATION AND TOLERANCE OF NICKEL AND ZINC IN *THLASPI CAERULESCENS* FROM SERPENTINE, CALAMINE, AND NORMAL SOIL

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1. Introduction

Thlaspi caerulescens is a well-known hyperaccumulator of heavy metals, particularly Ni or Zn, the latter sometimes in combination with Cd and Pb. Under natural conditions, this species exhibits a striking variation in foliar metal compositions. This may be due to differential soil metal compositions at the sites of plant collection, genetically based interecotypic variation, or a combination of both. Interecotypic comparisons under controlled experimental conditions have hardly been made thus far. Exploration of the nature and the degree of intraspecific genetic variation with regard metal to preference patterns for uptake, transport, and tolerance might be rewarding, from a phytoremediation viewpoint. In this study, we compared the uptake, transport, and tolerance of Zn and Ni, supplied either alone, or in factorial combinations, in *T. caerulescens* ecotypes from calamine, serpentine, and nonmetaliferous soil.

2. Materials and Methods

Seeds were collected at La Calamine (LC), Belgium (calamine soil), Monte Prinzera (MP), Italy (serpentine soil), Kuopio (Ku), Finland (normal soil), and Lellingen (Le) Luxemburg (normal soil). They were sown on moist peat and seedlings were transferred to a modified half-strength Hoagland's, amended with factorial combinations of $NiSO_4$ (0, 1, 10, and 100 μM) and $ZnSO_4$ (1, 10, and 100 μM). After three weeks of growth, during which the solutions were replaced twice per week, the plants were harvested, after desorbing the root systems with ice-cold 5-mM $PbNO_3$ (1 h).

Roots and shoots were dried at 80 °C, wet-ashed, and analysed for Zn and Ni, using AAS. Total uptake was calculated on a total plant dry weight basis. Shoot to root metal concentration ratios were used as a measure of root to shoot transport.

3. Results and Discussion

There were pronounced interecotypic differences in Zn and Ni tolerance. The patterns observed corresponded well with the soil metal compositions at the sites of seed collection. The normal soil ecotype (Ku, Le) was severely stunted and chlorotic at 100 μM Zn (both with and without Ni), and at 100 μM Ni (both with and without Zn). Plants from Kuopio even developed chlorosis at 10 μM Ni with 1μM Zn. They remained green in the 10 μM Ni + 10 μM Zn treatment, however, probably due to a Zn-imposed decrease of the Ni uptake (see below). Serpentine plants were stunted and chlorotic at 100-μM Zn (with and without Ni), but maintained normal growth and pigmentation in the 100-μM Ni treatments. Calamine plants did not suffer from the 100-μM Zn treatments, but developed slight chlorosis in the 100-μM Ni treatments (with and without Zn).

All ecotypes hyperaccumulated Zn, albeit at strongly different rates. Calamine plants accumulated much less Zn than the others, particularly at higher external concentrations. In either ecotype, the Zn accumulation rates were almost unaffected by the Ni concentrations in the nutrient solution. Ni hyperaccumulation occurred in the normal soil ecotype (Ku, Le) and,

particularly, the serpentine ecotype. Zn effectively reduced the accumulation of Ni. In the 10 μM Ni + 10 μM Zn treatment, for example, Ni uptake was less than 20% of that in the 10μM Ni + 1μM Zn treatment (Table 1). The calamine ecotype accumulated no more Ni than a nonhyperaccumulator species would have done, irrespective of the Zn concentration in the nutrient solution.
Zn root to shoot transport, as estimated from the shoot to root concentration ratios, was much higher in the nonmetallicolous ecotype (Ku, Le) than in the metalicolous ones (LC, MP). The same appeared to hold true for Ni transport. Zn transport was strongly inhibited by Ni, and Ni transport by Zn, though only in the nonmetallicolous ecotype. In the metallicolous ecotypes, there was no detectable mutual or one-sided inhibition.

Table 1: Ni uptake (± SE) (μmol g^{-1} total plant dry weight) in *T. caerulescens* populations grown for 3 weeks at 10 μM Ni in the nutrient solution, in combination with 1, 10, and 100 μM Zn.

	Zinc concentration (μM)		
Population	**1**	**10**	**100**
La Calamine	1.51 (0.10)	1.73 (0.64)	1.87 (0.43)
Monte Prinzera	48.30 (7.32)	10.00 (1.44)	3.61 (0.43)
Kuopio	11.78 (0.65)	1.93 (0.36)	nd
Lellingen	**17.30 (2.25)**	**2.92 (0.40)**	**nd**

nd = not determined (plants were stunted and chlorotic)

4. Conclusions

1) Metallicolous *T. caerulescens* ecotypes are specifically adapted to their native soils. Calamine plants exhibit increased tolerance to Zn and, though to a lower degree, Ni, associated with reduced rates of uptake and transport of these metals. Serpentine plants specifically exhibit Ni tolerance, associated with increased, rather than reduced uptake, and decreased transport of Ni and Zn.
2) At least two different uptake systems seem to be involved in Zn/Ni hyperaccumulation in *T. caerulescens*, viz. (i) a highly Zn-specific high-affinity system, and (ii) a low-affinity Zn/Ni uptake system, which strongly prefers Zn over Ni. The second one seems to be suppressed in the calamine ecotype, but overexpressed in the serpentine ecotype.
3) Competitive interactions of Ni and Zn with regard to root to shoot transport are evident in the nonmetallicolous ecotype, but not in the metallicolous ones. This may be taken to point at the existence of differentially expressed plant-internal transport systems with distinct metal preferences.

ZN ACCUMULATION BY CARDAMINOPSIS HALLERI AS A FUNCTION OF INITIAL METAL SOURCE IN DIFFERENT SUBSTRATES

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1. Introduction

According to BAKER and WALKER (1990), *C. halleri.* is a hyperaccumulator of Zn and Cd. However little is known about its growing conditions on polluted soils. Preliminary field study of a metallophyte grassland in the northern part of France (BALABANE et al., 1999), showed that *C. halleri.* hyperaccumulate Zn and Cd in its shoots (DAHMANI-MULLER et al., 1998). Studies on Zn accumulation by *C. halleri.* under controlled conditions were initiated in order to answer the following questions : *(i)* is Zn absorption and accumulation by *C. halleri.* influenced by the initial metal source, *(ii)* is there any correlation between the total or extractable Zn contents in soils and Zn contents in *C. halleri.*?

The main objective of this work was to study the ability of *C. halleri.* to grow on various polluted soils and to assess its applicability for phytoremediation use.

2. Materials and Methods

Different types of metal pollutant specimen were mixed with a non polluted loamy soil in order to produce initial substrates in which metals are associated to various soil constituents. Two series of such substrates were prepared, one at a final Zn content of 500, the second at 2000 mg.kg^{-1}. Identical N (66 mg.kg^{-1}), P (50 mg.kg^{-1}) and K (245 mg.kg^{-1}) fertilisations were added. Different metal pollutant samples were collected at a former zinc smelter site and in different horizons of the metallophyte grassland : *(i)*Two types of ground industrial slag (S1084 and S1085) with different proportions of Zn, Pb, Cd, *(ii)* litter (OL horizon), *(iii)* the organo-mineral horizon (Ah), *(iv)* the clay fraction extracted from the underlying, former ploughed L horizon and *(v)* Zn as Zn SO_4, $7H_2O$ (Zn).

Seeds of *C. halleri.*, collected on the metallophyte grassland, were sown on compost. After 3 weeks, 4 seedling per pot were planted out.

Total and 0.01M. $CaCl_2$ extractable Zn, Pb, Cd, Cu, P, S were determined on the substrates before plant bedding and after harvesting plants. 2 months later, plants were harvested, the shoots were cut and roots were separated from the soil.

The dry weight of shoots was measured. Total content of Zn, Pb, Cd, Cu and P in shoots and roots was determined by standardised methods (AFNOR, 1987). All analyses were performed in 4 replicates.

3. Results and Discussion

Soil analyses show that total Zn contents of the 2000 mg.kg^{-1} series vary from 2052 to 1238 mg.kg^{-1}. Extractable Zn amount varies from 5% to 48% according to the substrates (Table 1).

Table 1 : Total and extractable metals concentration in initial substrates **(mg.kg^{-1} dry soil)**

Metal source	**$ZnSO_4$**		**Clay fraction**		**Litter (OL)**		**Slag 1085**		**Slag 1084**		**Ah horizon**		**Control**
Target concentration	**500**	**2000**	**500**	**2000**	**500**	**2000**	**500**	**2000**	**500**	**2000**	**500**	**2000**	
Total Zn (T)	558,4	2052,8	357,3	1238	409,4	1746	438,6	1621,1	304,3	1642	368,9	1485	64
EXTRACTABLE ZN (E)	147	992	36,8	228	55,9	187	11,3	151	14,9	124	18,04	75,8	0,263
Zn(T) / Zn(E)	*26,32*	***48,32***	*10,29*	***18,41***	*13,65*	***10,71***	*2,57*	***9,31***	*4,89*	***7,55***	*4,89*	***5,10***	***0,410***
Total Pb (T)	62,9	70,4	112,6	214,4	103,4	316,9	446,5	1690,7	499,5	2181,1	150,6	461,4	62,2
Extractable Pb (E)	<0,01	<0,01	<0,01	0,039	0,018	0,145	0,13	*4,831*	0,051	1,32	0,015	0,097	<0,01
Pb(T) / Pb(E)				0,018	0,017	0,045	0,029	0,285	0,010	0,060	0,0099	0,021	
Total Cd (T)	0,24	0,27	3,61	13,52	2,87	13,22	11,76	50,29	1,15	2,68	1,32	4,66	0,292
Extractable Cd (E)	0,041	0,069	0,563	3,195	0,4	1,386	2,124	20,6	0,056	0,18	0,099	0,438	0,014
Cd(T) / Cd(E)	17,08	25,55	15,59	23,63	13,93	10,48	18,06	*40,96*	4,86	6,71	7,5	9,39	4,79

M(T) / M(E) : percentage of metal extractable by 0.01 M. $CaCl_2$

3 groups of substrates with different amounts of extractable Zn can be distinguished : a) in the $ZnSO_4$ amended substrate almost 50% of the added Zn is extractable; b) in the clay fraction amended substrate approximately 20% of the added Zn is extractable c) in the substrates, amended by litter, by slag or by organic matter from the Ah horizon, extractable Zn amounts vary between 10 and 5% (Table 1).

Table 2 : Dry matter (g), produced by Cardaminopsis halleri grown on different substrates.

substrates	**Zn 2000**	**Clay 2000**	**OL 2000**	**Ah 2000**	**Ah 500**	**Zn 500**	**S1085 500**	**Clay 500**	**OL 500**	**S1084 500**	**S1084 2000**	**control**	**S1085 2000**
dry matter/pot	1,93	1,92	1,88	1,83	1,73	1,66	1,52	1,48	1,47	1,46	1,24	1,24	0,305
standard deviation	0,108	0,057	0,258	0,085	0,059	0,088	0,100	0,102	0,290	0,208	0,216	0,106	0,278

Maximum dry matter production is observed for *C. halleri.* grown on the 2000 series, except for the two slag amended substrates (Table 2). No correlation is observed between *C. halleri* dry matter production and the amounts of extractable Zn.

4. Conclusions

C. halleri can grow on substrates containing different amounts of Zn from different sources. In a first approximation, the amount of 0.01 M. $CaCl_2$ extractable Zn is not related to *C. halleri* growth. On substrates amended with fine ground slag for *C. halleri* growth is decreased (to zero), especially for S1085.

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COMPARATIVE ANALYSIS OF METAL UPTAKE, TRANSPORT AND SEQUESTRATION IN HYPERACCUMULATOR PLANTS

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1. Introduction

Hyperaccumulator plants are among the most metal-tolerant species in the plant kingdom. Several hundred species are known, some of which can accumulate Mn, Ni, or Zn to more than 1 % of shoot dry biomass (Baker and Brooks, 1989). Such plants must have effective mechanisms for metal trafficking at a number of levels, including (a) metal chelation in the cell cytoplasm to provide a biochemical basis for tolerance, (b) long-distance transport of the metal from root to shoot, and (c) sequestration of metals at high concentrations in the vacuole of shoot cells.

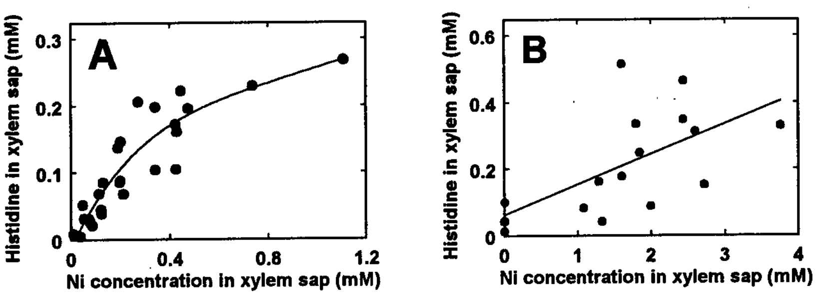
In earlier studies with nickel-hyperaccumulator plants, we discovered that species of *Alyssum* (Brassicaceae) produce free histidine as a specific and proportional response to nickel exposure (Krämer *et al.*, 1996). Histidine appeared to be important both as a high-affinity ligand for nickel detoxification and in facilitating nickel transport out of the root in the xylem. Here, we have addressed two further questions concerning this response. First, is the histidine response found in nickel-hyperaccumulator plants outside the genus *Alyssum*? Second, does hyperaccumulation of other metals besides nickel induce production of free histidine?

2. Materials and Methods

All plant material used in this study was grown hydroponically using modified 0.1-strength Hoagland solution No. 1 with EDDHA as the Fe-chelator. Seed material was kindly donated by K. Balkwill (*Berkheya coddii*), T. Jaffré (*Grevillea exul* var. *exul*) and M.R. Macnair (*Cardaminopsis halleri*). Metals were quantified by atomic absorption spectrophotometry. Amino acids were analysed by reverse-phase HPLC, and metal coordination by extended X-ray absorption fine structure (EXAFS) analysis at the U.K. Synchrotron Radiation Source at Daresbury, both as described by Krämer et al. (1996).

3. Results and Discussion

As found previously for *Alyssum bertolonii*, *A. lesbiacum* and *A. murale*, exposure of *A. pintodasilvae* to nickel elicited a proportional increase in free histidine in xylem sap collected as root-pressure exudate (Fig. 1A). However, histidine concentrations tended to saturate at the highest nickel concentrations, possibly reflecting the slightly lower degree of nickel tolerance of *A. pintodasilvae* compared with the other *Alyssum* spp. In another known nickel-



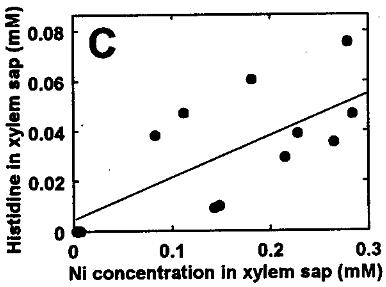


Figure 1. Relationship between nickel and histidine concentrations in the xylem sap of (A) *Alyssum pintodasilvae* ($r^2 = 0.838$, $P < 0.001$); (B) *Streptanthus polygaloides* ($r^2 = 0.440$, $P < 0.01$); (C) *Berkheya coddii* ($r^2 = 0.609$, $P < 0.01$).

hyperaccumulator species from the family Brassicaceae, *Streptanthus polygaloides,* we also observed a significant positive correlation between xylem nickel and histidine concentrations (Fig. 1B). Similarly, in the nickel hyperaccumulator *Berkheya coddii* (family Asteraceae), a positive correlation was observed between xylem nickel and histidine (Fig. 1C). EXAFS spectroscopy also provided evidence for nickel coordination with histidine *in planta* in *B. coddii.* Thus, the histidine response is observed not only in the Brassicaceae but also in a separate, phylogenetically distant family within the angiosperms. In contrast, no significant correlation was observed between Mn and histidine concentrations in the xylem of the manganese hyperaccumulator *Grevillea exul* var. *exul* (Proteaceae), or between Zn and histidine in the zinc hyperaccumulator *Cardaminopsis halleri* (Brassicaceae).

4. Conclusions

The histidine response observed in nickel-hyperaccumulator plants appears to have evolved more than once within the plant kingdom. The response, however, has not been observed in hyperaccumulators of Mn or Zn, and so seems to be intrinsically specific for Ni. The ways in which Ni is transported and sequestered within plant, and the effect on metal accumulation of co-contamination of the root medium with organic pollutants, are currently under investigation.

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PHYTOVOLATILIZATION AND THE PHYTOREMEDIATION OF SELENIUM

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1. Introduction

Selenium (Se) can present a very serious environmental problem, especially in the western United States where drainage water from agricultural irrigation contains Se as a natural contaminant (soils accumulate Se as a result of the weathering of Se containing shale rocks). Phytoremediation is a low-cost and environmentally friendly way of cleaning up Se in polluted soils and waters (Terry and Zayed, 1998). Plants may take up and immobilize Se in their tissues (*phytoextraction*). In addition, plants can remove Se from contaminated substrates and transform it into volatile forms, e.g., dimethylselenide. This has been termed *phytovolatilization* (Terry and Zayed, 1998). Our goals are to understand the mechanisms controlling Se assimilation and volatilization in plants, and plant/microbe associations, and to develop these phenomena into a practical method for the cleanup of Se from the environment.

2. Materials and Methods

Our studies include wetland ecology, plant physiology and biochemistry, microbiology and molecular biology.

3. Results and Discussion

Constructed wetlands: Constructed wetlands can be used to clean up Se from large volumes of industrial wastewater. Research conducted at the Chevron oil refinery in California demonstrated that 89 percent of the Se from oil refinery wastewater was removed by a 36-hectare constructed wetland that processed approximately ten million liters of oil refinery effluent per day; as much as 10 to 30 percent of the Se was removed by volatilization to the atmosphere (Hansen et al., 1998). Selenium removed by volatilization cannot enter into the local food chain where it may become toxic to wildlife and humans, and eliminates the need for disposal of hazardous plant waste. In a second constructed wetland project at Corcoran, California, we built ten quarter-acre wetland cells (planted with different wetland plant species) to test the idea that flow-through constructed wetlands may be used to remove Se from agricultural drainage water. The wetland cells significantly reduced the levels of Se (in some instances from 25 to <5 ppb Se), as well as strontium and vanadium, from the inflow.

Advances in the biochemistry of selenium assimilation: Selenium biochemistry is similar in many respects to the biochemistry of sulfur. We have developed a biochemical pathway for the uptake, assimilation and volatilization of Se that proposes that selenate is absorbed and assimilated in a manner similar to sulfate (Zayed and Terry, 1992). Using a molecular biology approach, we determined whether transporters and enzymes used in sulfate absorption and assimilation are also used in the absorption and assimilation of selenate. The reduction of sulfate to sulfite in plant tissues is carried out by ATP sulfurylase. We were the first to demonstrate in vivo that this enzyme also catalyses the reduction of selenate (Pilon-Smits et al., 1999). Furthermore, we identified this enzyme as a major rate-limiting step in the assimilation of selenate by plants to organic Se (de Souza et al., 1998; Zayed et al., 1998). Both of these findings were obtained by expressing genes for ATP sulfurylase in Indian mustard and testing

the resulting transgenic plants using high energy x-ray absorption spectroscopy (XAS) speciation to show that overexpressing the enzyme led to the metabolic conversion of selenate to organic forms of Se (e.g., selenomethionine).

Role of rhizosphere bacteria: We have investigated the role of bacteria in the volatilization of Se by plants. We found that rhizosphere bacteria promote the plant uptake of selenate (de Souza et al., 1999). Part of this stimulatory effect was due to a bacterially-mediated increase in root hair development which increases the root surface area available for Se uptake. More importantly, we found that rhizosphere bacteria stimulate the production of a heat-labile compound (probably a peptide), which facilitates the transport of selenate across the plasma membrane into the root (de Souza et al., 1999). When this heat-labile compound was added to the rhizosphere of axenically-grown plants, it resulted in a substantial accumulation of selenate. Compared to axenically-grown controls, sterile plants inoculated with rhizosphere bacteria had a 5-fold higher Se concentration in their roots. Furthermore, the inoculated plants had a 4-fold higher rate of Se volatilization, probably because of the microbially-induced increase in Se uptake.

4. Conclusions

Selenium-contaminated industrial and agricultural wastewater can be remediated using constructed wetlands. Genetic engineering of Indian mustard (overexpression of the gene for ATP sulfurylase) resulted in transgenic plants that accumulated, tolerated, and volatilized more Se than the wildtype. Rhizosphere microbes were shown to enhance the plant uptake of Se, which in turn increased the rate of Se volatilization.

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PROGRESS TOWARDS A MOLECULAR UNDERSTANDING OF METAL HYPERACCUMULATION IN PLANTS

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Project Summary

The majority of plants that occur on metalliferous soils are known to exclude toxic metals from their shoots (Baker and Walker, 1990). A number of species, however, have developed an unusual adaptation to metal-rich soils. Instead of excluding toxic metals, so-called hyperaccumulators accumulate metals such as Ni, Zn or Se in their above-ground biomass. This trait is highly attractive for the development of technologies aimed at the decontamination of metal-polluted soils using plants (Chaney, 1983; Salt *et al.*, 1998). Nickel concentrations in field-collected leaves of Ni hyperaccumulators are typically above 0.1% (w/w) in dry biomass, and concentrations as high as 3.8% Ni in dry biomass have been reported (Baker and Brooks, 1989), whereas Ni concentrations in the surrounding vegetation usually range between 0.3 and 100 ppm (Brooks and Dudley, 1987). *Thlaspi goesingense* Hálácsy occurs on Austrian Ni-rich ultramafic (serpentine) soils where it has been reported to hyperaccumulate Ni (Reeves and Brooks, 1983). We investigated Ni tolerance and Ni uptake into roots and shoots in *T. goesingense* and a weedy population of the related non-accumulator *Thlaspi arvense* L. Our aim was to assess the roles of metal tolerance and metal transport in the hyperaccumulator phenotype by comparing *T. goesingense* with *T. arvense*

As long as both species were unaffected by Ni toxicity the rates of Ni translocation from roots to shoots were the same in the hyperaccumulator and the non-accumulator (Krämer et al., 1997). It was the Ni-sensitivity of the non-accumulator *T. arvense* which caused the breakdown of Ni uptake and therefore reduces long-term accumulation. Our data suggest that Ni tolerance is sufficient to explain the Ni hyperaccumulator phenotype observed in hydroponically cultured *T. goesingense* when compared with the Ni-sensitive non-hyperaccumulator *T. arvense* (Krämer et al., 1997).

Because of the central role Ni tolerance plays in the ability of the hyperaccumulator *T. goesingense* to maintain efficient root-to-shoot Ni transport and accumulation we investigated the basis of this tolerance. Exposure to Ni of leaf protoplasts isolated from both *Thlaspi* species demonstrated the existence of a tolerance mechanism operating at the cellular level in the hyperaccumulator *T. goesingense*. Upon exposure to Ni for 24 h, the viability of protoplasts isolated from *T. arvense* was significantly reduced to about two thirds of the unexposed controls. In *T. goesingense*, cell viability of Ni-exposed protoplasts was slightly higher than that of the unexposed controls, suggesting that the protoplasts were more Ni-tolerant (Krämer et al., 1997).

However, the biological basis of this cellular Ni tolerance mechanism is unknown. We have investigated the role of vacuolar compartmentalization in this tolerance mechanism. A direct comparison of the Ni contents of intact vacuoles from leaves of *T. goesingense* and the non-tolerant non-accumulator *T. arvense* revealed that the hyperaccumulator has an enhanced capacity to accumulate Ni within leaf vacuoles. In fact 53% of the intracellular Ni was found within the vacuole of the hyperaccumulator, approximately 2 fold more than in the non-

accumulator (Table 1), after exposure to 1 μM Ni for 1 day (conditions that were non-toxic to the non-accumulator). This supports the hypothesis that the vacuole plays an important role in the cellular Ni resistance mechanism in the hyperaccumulator. At present we are investigating the molecular mechanisms involved in this vacuolar Ni compartmentalization.

Table 1. Nickel distribution between apoplast, vacuole and cytoplasm. Values are means of three experiments ± standard errors. Plants were exposed to 1 μM Ni for 1 day.

	Apoplast Ni	Vacuolar Ni	Cytoplasmic Ni
		% of total leaf Ni	
T. goesingense	66.7 ± 2.3	17.1 ± 3.3	16.2 ± 1.0
T. arvense	68.9 ± 4.3	8.3 ± 2.8	22.8 ± 2.5

It has recently been suggested that free histidine plays a role in Ni transport/tolerance in related hyperaccumulating *Alyssum* species (Krämer, 1996). To test this hypothesis in *Thlaspi* species we have cloned three key genes involved in histidine biosynthesis in *T. goesingense*, THG1 (GenBank AF003347), THD1 (GenBank AF023141) and THB1 (GenBank AF023140), encoding for ATP phosphoribosyltransferase (ATP PRT), imidazoleglycerol phosphate dehydratase (IGPD), and histidinol dehydrogenase (HD), respectively. We have isolated mRNA from *T. goesingense* exposed to Ni and we are now in the process of using THG1, THB1 and THD1 as probes to study the transcriptional regulation of these genes by Ni, using Northern analysis. We have also recently been successful at expressing THG1 in *E. coli* and are now in the process of purifying the recombinant His-tagged ATP PRT. Once purified, the activity of ATP PRT will be studied for allosteric regulation by Ni and histidine.

Acknowledgments
I would like to thank Dr. Ute Krämer for her hard work and patience while performing the Ni cellular localization studies, and Dr. Xianghe Yan for cloning the histidine biosynthetic genes. This research was supported by a NATO fellowship awarded to Dr. Krämer by the German Academic Exchange Service (DAAD), and by the US Department of Energy (grant no. DE-FG07-98ER20295) to Dr. Salt.

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A MOLECULAR PHYSIOLOGICAL ANALYSIS OF HEAVY METAL TRANSPORT IN A HYPERACCUMULATING PLANT SPECIES

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1. Introduction

Thlaspi caerulescens J&C Presl has been reported to have a great potential for the extraction of Zn from metalliferous soils (Reeves and Brooks, 1983; Brown et al., 1994; Brown et al., 1995). The identification of this and several other metal hyperaccumulator plant species demonstrates that the genetic potential exists for successful phytoremediation of contaminated soils. Despite its ability to accumulate high levels of zinc and other metals in the shoot, the use of *T. caerulescens* for commercial-scale soil remediation is severely limited by its small size and slow growth. The transfer of zinc-hyperaccumulating properties from *T. caerulescens* into high-biomass producing plants has been previously suggested as a potential avenue for making phytoremediation a commercial technology (Brown et al., 1995). Progress in this area, however, is hindered by a lack of understanding of the fundamental mechanisms involved in zinc transport and accumulation in roots and shoots.

We have been conducting an integrated physiological, biochemical and molecular investigation of Zn uptake, translocation and storage in *T. caerulescens*, compared with a related non-accumulator, *Thlaspi arvense* (Lasat et al, 1996, 1998). The findings presented in this talk will show that a number of Zn transport sites are stimulated in *T. caerulescens*, and at the molecular level, this stimulation involves an alteration in the regulation of Zn transporter genes by plant Zn status.

2. Materials and Methods

Plant Material. Seedlings of *T. arvense* and *T. caerulescens* were grown on a 1-mm nylon mesh floated on a nutrient solution containing the following macronutrients (mM): Ca^{2+}, 0.8; K^+, 1.2; Mg^{2+}, 0.2; NH_4^+, 0.1; NO_3^-, 2.0; PO_4^{3-}, 0.1; SO_4^{2-}, 0.2, and micronutrients (μM): BO_3^{2-}, 12.5; Cl^-, 50; Cu^{2+}, 0.5; Fe^{3+}-EDDHA, 10.0; MoO_4^{2-}, 0.1; Mn^{2+}, 1.0; Ni^{2+}, 0.1; Zn^{2+}, 1.0. The solution was buffered at pH 5.5 with 1 mM Mes-Tris. The mesh was covered with black polyethylene beads to minimize illumination of the growth solution. Seedlings were grown in a growth chamber at 24°C/15°C (light/dark: 16:8 h) under light intensity of 300 μmol photons m^{-2} s^{-1}. In order to use *T. caerulescens* and *T. arvense* seedlings at a similar developmental stage, experiments were conducted with *T. caerulescens* seedlings that were approx. 14-days older than *T. arvense* seedlings.

Radiotracer ($^{65}Zn^{2+}$) uptake experiments. Uptake experiments were conducted with roots of intact *Thlaspi* seedlings immersed in either 80 mls of an aerated uptake solution consisting of different concentrations of $ZnCl_2$, 2 mM Mes-Tris (pH 6.0) and 0.5 mM $CaCl_2$ in Plexiglas wells of an uptake system (for unidirectional ^{65}Zn influx studies) or in 1l pots (for long term Zn accumulation and translocation studies). Uptake was initiated by addition of $^{65}ZnCl_2$ (0.08 μCi/ml to each uptake well or pot to yield final total Zn concentrations between 0.5 and 100 μM. After the prescribed uptake period, the radioactive uptake solution was vacuum-withdrawn and roots desorbed in an ice-cold desorption solution containing 100 μM $ZnCl_2$, 5 mM Mes-Tris (pH 6.0) and 5 mM $CaCl_2$. for 15 min, seedlings were harvested, roots excised, blotted, weighed, and ^{65}Zn measured.

Cloning of Zn transport genes. A Zn transport cDNA was cloned via functional complementation of a yeast mutant defective in Zn uptake. A *Thlaspi caerulescens* cDNA library was constructed in the yeast expression vector, pFL61, and 300,000 yeast transformants were screened for growth on low Zn media. This approach allowed us to clone a 1.2 kb Zn transport cDNA, *ZNT1*, from *Thlaspi caerulescens*. Standard methods were used to conduct Southern and Northern analysis for *ZNT1* with DNA and RNA isolated from roots and leaves of the two Thlaspi species. Also, the transport characteristics of the ZNT1 protein were studied via ^{65}Zn influx studies in yeast expressing *ZNT1*.

2. Results and Discussion

The physiological examination of Zn^{2+} influx in the two Thlaspi species showed that there were a number of Zn^{2+} transport sites altered in *T. caerulescens* compared to *T. arvense* that contribute to the Zn hyperaccumulation. These include: 1) stimulated Zn influx across the root cell plasma membrane; 2) altered root tonoplast transport in the two Thlaspi's, such that *T. arvense* sequesters more Zn in the root vacuole; 3) higher levels of Zn^{2+} in *T. caerulescens* xylem and 4) increased leaf Zn^{2+} uptake in *T. caerulescens*.

Subsequently, we investigated the molecular mechanisms underlying these alterations in Zn transport. A functional complementation approach allowed us to clone a 1.2 kb Zn transport cDNA, *ZNT1*, from *T. caerulescens*. Sequence analysis of *ZNT1* revealed that it shares significant homology with *IRT1*, a putative Fe transporter in Arabidopsis, and *ZRT1*, a yeast high affinity Zn transporter. Characterization of the concentration-dependent transport properties of ZNT1 expressed in yeast revealed that it encodes a system that mediates saturable Zn^{2+} uptake as well as non-saturating Cd^{2+} uptake. Northern analysis of *ZNT1* demonstrated that it is expressed at very high levels in roots and shoots of T. caerulescens and its expression is not influenced by plant Zn status. *ZNT1* transcript abundance was dramatically lower in the non-accumulator, *T. arvense*, and expression in this species was stimulated by Zn deficiency. These findings indicate that Zn hyperaccumulation in *T. caerulescens* is due in part to an alteration in the regulation of root and shoot Zn transporters by plant Zn status, resulting in enhanced expression of the gene encoding this transporter.

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COPPER RESPONSES IN THE MODEL PLANT *ARABIDOPSIS THALIANA*

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Copper, an abundant transition metal, is an essential micronutrient for all living organisms. In plants, copper plays a vital role in both photosynthetic and respiratory electron transport, and it functions as a cofactor in a variety of enzymatic reactions. While the redox activity of copper makes it an ideal cofactor in cellular energy transfer functions, it also gives the ion the potential to cause tremendous oxidative damage if intracellular concentrations become too high. Homeostatic mechanisms have therefore evolved to regulate the concentration of free copper ions in the cell.

1. Metallothioneins and Non-Protein Thiols

Inspired by studies in animals and yeast, a good deal of attention has been paid to the role of metallothioneins (MTs) and non-protein thiols (NPTs) in the copper ion homeostasis of plants. In our lab, an attempt was made to determine whether a correlation could be found between growth inhibition and either NPT levels or the expression of MT mRNA. MT1 was expressed at the basal rate while MT2 was copper inducible in all 10 ecotypes. MT2 expression correlated with root growth while MT1 and NPTs did not.

To determine if MT mRNA expression reflects protein levels, MT 1 and 2 proteins were identified with polyclonal anti-MT antibodies. Protein accumulation patterns reflected mRNA expression, although protein levels were quite low. Subsequently, MT1, MT2, and a novel 7 kDa protein, MT3 were identified by peptide sequencing. The MT3 cDNA sequence was subsequently obtained.

In a further study of patterns of expression of MT proteins, antisense mutants of MT1a and MT2a provided by Dr. Peter Goldsbrough were assayed for copper sensitivity in root growth assays. At 36h the growth of all of the mutants was more inhibited than the controls, with the MT2 antisense mutants showing the greatest levels of inhibition. These results are consistent with the correlation of MT2 mRNA expression and seedling tolerance described previously.

2. Copper effects on membrane integrity

Mechanisms of K+ Efflux

To study the relationship between copper sensitivity and membrane permeability, seedling growth was compared with potassium efflux and copper uptake. Inhibition of root extension in 40 μM copper correlated well with potassium efflux (R=0.96) and a decrease in tissue osmolality (R=0.96) at 36h, but not at 4h. In detailed time course studies, both more and less sensitive ecotypes showed decreased retention of potassium initially, but potassium levels were subsequently restored only in less sensitive ecotypes. In time course measurements, the copper contents of the four ecotypes were not significantly different when normalised on a fresh weight basis. We conclude that differences in seedling metal tolerance are not caused by constitutive differences in membrane permeability but, rather, by differences in the ability to maintain membrane integrity.

To probe the mechanism of the early phase of K^+ efflux, we have tested the ability of a variety of channel blockers and inhibitors on both copper and peroxide-induced K^+ efflux. The K^+ channel blockers tetraethylammonium chloride (TEA) and 4-aminopyridine (4-AP) both

inhibited copper-induced K^+ efflux, but only 4-AP inhibited peroxide-induced leakage. Copper induced lipid peroxidation of the roots did not begin until after 4 hours of treatment. We conclude that short-term K^+ efflux in response to copper is due to the activation of TEA- and 4-AP-sensitive K^+ efflux channels, rather than to the formation of nonspecific holes in the membrane due to lipid oxidation.

Membrane Lipids

When both mRNA and protein levels of low molecular weight copper-induced copper-binding proteins were monitored in leaves, two proteins ostensibly involved in lipid biosynthesis, a novel 6kDa ACP-like protein (6KACL) and the acyl coA binding protein (ACBP), as well as a 12 kDa copper homeostasis protein (ATX1) and a plastid DNA repair protein (DRT112) were identified. Leaf growth of 35S:ACBP antisense mutants obtained from Dr. Ljerka Kunst was inhibited as much as 70% by 20μM copper treatment. In contrast, the growth of one overexpression line was stimulated by 30-40% relative to the controls. Induction of C22 fatty acids and sterols by copper were also reduced in antisense transformants. Changes in membrane composition may be a factor in the copper recovery response, especially in leaves.

ECOTOXIC MERCURY REDUCTION BY TRANSGENIC PLANTS

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1. Introduction

Over the last half century mercury pollution has been recognized as a devastating environmental hazard. The catastrophe in Minamata Bay, Japan demonstrated the deadly consequences of mercury waste discharge, causing more than a thousand deaths and a prolonged and expensive cleanup. This mishap led industrialized nations to sharply curtail mercury waste and to seek alternatives to its use in manufacturing or agricultural applications. However, many sites remain highly contaminated and, in some developing countries, mercury containing wastes continue to be generated. Industrial mercury discharges are typically in the form of highly toxic, ionic mercury [Hg(II)], which can be converted by anaerobic bacteria to methylmercury (MeHg). Of the three principle mercury forms, MeHg is the most ecologically hazardous due to its tendency to biomagnify to high levels in animal tissues at higher levels in the food web. Bacteria have evolved a biological approach for the detoxification of mercurials allowing them to colonize contaminated sites by enzymatic conversion of MeHg and Hg(II) to much less toxic, volatilized Hg(0) (Fig. 1). We have used a molecular genetic approach to transfer the bacterial mercury resistance genes to a variety of plant species as a potential means for environmental mercury phytoremediation.

MeHg --(MerB)--> Hg(II) --(MerA)--> Hg(0)

Figure 1: Shown are the enzymatic reactions encoded by the bacterial *merA* and *merB* genes, with the final product, Hg(0).

2. Materials and Methods

The bacterial genes encoding organomercurial lyase (MerB) and mercuric reductase (MerA) were manipulated and transferred to a variety of plant species using standard methods for transgenic plant development (Rugh et al., 1996; Rugh et al., 1998).

Plants transformed with *mer* genes were tested for resistance to toxic levels of mercury on a variety of substrates spiked with organic or ionic mercurials. Agar-based gel media was amended with either Hg(II) for *merA* plants or MeHg or phenylmercuric acetate (PMA) for *merB* plants and used in seed germination and survival assays. Soils were spiked to a range of Hg(II) concentrations (0, 100, and 500 ppm) and used for plant growth and soil-interaction evaluations (Heaton et al., 1998). Transgenic and control tobacco plantlets (1-2 cm height) were transplanted to pots containing either a commercial potting soil or each of two field soils. They were maintained for a period of one month and then plant survival and biomass data were collected.

Mer-plants were analyzed for their ability to remove mercurial compounds from spiked and contaminated media, including agar-gels, aqueous hydroponics, and soils. Arabidopsis and tobacco plantlets were placed in reaction tubes containing aqueous solutions of either Hg(II) or PMA with volatilized Hg(0) measured directly from the reaction vessels in short assays (~5-20 min). *MerA*-tulip tree plantlets were placed in tubes containing agar-gel spiked with Hg(II) and

sampled periodically for Hg(0) release over a one week period. Larger-scale assays were conducted using tobacco plants grown in Hg(II)-spiked hydroponic medium in 1 L vessels with periodic analysis of tissue and solution Hg(II) content. Growth room and greenhouse studies were performed to evaluate the ability of *merA* plants to stabilize Hg(II) effluent loss and reduce the Hg(II) content of contaminated soils by plant uptake and phytovolatilization.

3. Results and Discussion

Expression of *mer* genes by transgenic plants conferred resistance to both organic and inorganic mercurial compounds. All seeds transformed with *merA* genes germinated and grew vigorously on agar media at higher Hg(II) concentrations (up to 20 ppm) than those shown to be lethal to nontransgenic seeds (~5 ppm). Similarly, *merB* transformed plants prospered at normally toxic levels of the organomercurials, MeHg and PMA (0.1 to 1 ppm). Seeds of plants having both genes were able to germinate and grow at even higher levels of organomercurials (~2 to 4 ppm). The relative levels of each of the mercury forms required for seed lethality show the higher toxicity of the organic compounds relative to Hg(II), as has been reported in animal assays.

Plants containing *mer* genes were able to release dramatically higher levels of Hg(0) from spiked growth medium than control plants. In short-term assays, *merA*-Arabidopsis reduced Hg(II) to Hg(0) at ~4-fold the rate relative to control plants, confirming the expected enzymatic mechanism for Hg(II) resistance. Arabidopsis plants engineered with both *merA* and *merB* genes successfully converted PMA to Hg(0), while plants having only one or no *mer* genes produced no detectable Hg(0). *MerA*-tulip tree plantlets were capable of greater than 10-fold the level of Hg(0) release from Hg(II)-spiked gel medium relative to nontransgenic control plantlets. In hydroponic assays, *merA*-tobacco plants removed 70-95% of Hg(II) from the system after 7 days in contrast to nontransgenic plants which removed only 20-25%.

MerA transformed tobacco plants had higher rates of survival and growth than control plants on all tested Hg(II)-spiked soil types. The commercial potting soil was extremely rich in organic matter and fertilizer and allowed survival for all plantlets. However, the *merA*-plants gained 1.5-fold and 4-fold more shoot biomass in the 100 ppm and 500 ppm commercial soils, respectively, than did the nontransgenic plants. The spiked field soils were more toxic than the commercial soils with high mortality among the control plants (75% on the 100 ppm, 100% on the 500 ppm), though less so for the *merA*-plants (10% on the 100 ppm, 50% on the 500 ppm). Growth of the surviving control plants was completely inhibited on spiked field soils, while *merA*-plants only showed stress when growing on the 500 ppm soils with about 30% biomass accumulation relative to 0 or 100 ppm pots. Analysis of soil Hg(II) content showed no significant differences among any of the soil x genotype treatments, most likely due to the brevity of the growth period.

4. Conclusions

This research has demonstrated that transfer of bacterial mercury resistance genes to plants gives them the ability to grow on normally toxic, mercury-containing substrates and also to mobilize reactive mercury from the medium by conversion to volatilized and detoxified Hg(0). This has great potential as a method to remove hazardous, bioavailable mercurials from contaminated environments hastening their recovery. Furthermore, plant-based detoxification and mobilization may be further manipulated to promote retention of Hg(0) as Hg(II) in plant shoot tissues, allowing sequestration to be an additional option for mercury phytoremediation.

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Fate of Radionuclides
(Special Symposium 2)

RADIOECOLOGY AND SOIL SCIENCE

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1. Introduction

The International Union of Radioecologists endeavours to promote a better assessment of the behaviour of radionuclides in the environment. Nuclear accidents of different nature have strongly influenced the views and scope of radioecology. A prime objective of radioecology is to understand how the interaction between radionuclides and the environment affects radiation dose. Such an understanding permits the second objective, which is to provide a suitable environmental management and rehabilitation methodology to reduce radiation dose with respect of the ecological and economic value of contaminated land.

2. Discussion

In the European radioecological world research aims at deciphering mechanisms at work at the micro-levels such as plant-soil interactions, plant accumulation etc. Research aims also to describe the mechanisms at work in whole ecosystems. Both levels are intimately connected, the results of the work at the micro-level having their bearings on the macro-levels. Soil science is a typical example where the research on the problems of Caesium persistence in some areas in Europe have definitely broadened the understanding of clay chemistry (Wauters et al. 1994). In many soils a significant proportion of the radionuclides would become fixed within the soil matter and unavailable for plant uptake or for mobilisation. This has its consequences for the radioecological half-life of the radionuclide. This has forced radioecologists concerned with dose assessments to link the micro-level findings on soil bio-availability mechanisms with macro-level phenomena on ***fluxes*** of radionuclides (Desmet 1991). Fluxes are calculated by multiplying the activity concentration of a radionuclide in a product by the total quantity of a product produced over time, with units of $Bq.t^{-1}$ .This approach imposes reflection into the direction of "dynamic models". Traditionally soil-plant transfers have been expressed by concentration ratios (Frissel 1989). This has been very useful, although undoubtedly it misses in its basic principle the time-dependency of the phenomena. The establishment of time dependencies is paramount for flux calculations. The flux approach is essential for the estimation of the relative importance of any ecosystem contribution to the dose to Man, and for the computation of its radionuclide "critical load"(Howard et al. 1996). This allows to identify the sites for interventions after an accident and the appreciation of their long-term effect . It also allows the assessment of the effect of any environmental management practice on the long term. An understanding of basic mechanisms at the soil level of the fate of radionuclides permits to better delineate countermeasure methodologies such as potassium application, use of manure, use of binding components such as some clays, some zeolites etc. From the above, the dependency but also the effect on other scientific disciplines must come into view. The Caesium persistence e.g. in especially "semi-natural" soils, high in organic matter, has been quite a problem after the Chernobyl accident. Exhaustive investigations have now virtually brought the solution and essential insights. The relative role of illite, vermiculite and organic matter is almost elucidated, and a new mechanistic terminology and adequate methodology set up. In a radioecological context two levels of users of the scientific assets can be identified. "Internal" user groups come forward when direct use is made by practitioners such as field engineers, foresters, farmers, and "external" users appear where the soil "Transfer Capacity" for radionuclides is connected to

"Ecosystem Vulnerability" (Howard et al. 1996). This terminology proposes to quantify the capacity of any ecosystem to induce a "critical load" and a dose to Man. This concept is paramount for the development of specific environmental Decision Support Systems (Burrough et al 1997). Such systems could assist both decision makers and field engineers.

3. Conclusions

In radioecology, soil science with its specific fields of research like speciation, bio-availability and mobility is an essential part of the work. The knowledge gathered serves purposes of dose calculations, and of purposes of environmental management through soil amendments. Its concepts are valuable for related basic research disciplines, and for related environmental management of heavy metal pollutants, mixed pollutants etc. Its property to be of a problem solving nature draws from a number of related disciplines including soil microbiology, soil mechanics, soil chemistry etc., and has the virtue to add to their interdisciplinarity.

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THE UIR ACTION ON 'ENVIRONMENTAL MODELS AND DATA'

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1. Background to the Action Group

Assessments of the consequences of releases of radionuclides to the environment rely on the application of mathematical models describing the transport of radionuclides from their source to man. Such models incorporate a wide range of environmental data derived from many experimental and field investigations on a variety of temporal and spatial scales. For many combinations of environmental conditions and radionuclide there is a consensus on the models and data that should be applied for a specific purpose. For others there is less consensus. In this context UIR established an Action Group with financial assistance from the European Commission to identify those combinations of system and radionuclide for which information or understanding was limited.

2. Action Group activities

Existing models were evaluated and data for the distribution and transport of radionuclides in the environment were collated. Since the remit of the Action Group was very wide it was necessary to focus it's activities on those topics which were currently considered as controversial. Relevant topic areas were identified by evaluating responses to a questionnaire which had been widely distributed within UIR. The Group then focussed it's attention on the following.

- The transfer of radionuclides to fruits.
- The role of microorganisms in the transfer of radionuclides in terrestrial food chains.
- Radioecological sensitivity.
- The role of soil fauna in the transfer and bioavailability of radionuclides in soil.
- The significance of particulate-associated radionuclides.
- Special radionuclides (actinides, low-energy beta emitters).
- Sea to land transfer.
- Environmental consequences of countermeasures.
- Effects of climate on foliar and root uptake of radionuclides.

This paper provides an introduction to the work of the Action Group and a summary of its conclusions.

THE ROLE OF SOIL FAUNA IN RADIONUCLIDE TRANSPORT IN SOILS

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1. Introduction

While the effects of anthropogenic substances, like industrial pollutants, agro-chemicals or radionuclides, on soil animals after deposition to soils have been subject to various investigations, much less attention has been paid to the inverse approach: The effect of soil fauna actions on the redistribution and biochemical conversion of those substances in soils. This was found in an analysis performed in the framework of a joint UIR-EURADOS-EULEP Action supported by the EC. Therefore, this topic was dealt with in a subtask trying to evaluate existing information to the point where it can be decided, whether or not this aspect is important enough to deserve consideration in radioecological models and whether or not more experimental research is needed.

2. Main Actions Of Soil Fauna

Soil fauna are commonly understood as the total population of animals living exclusively or at certain stages of their life in soil, and they are grouped in four size categories, ranging from microfauna (<0.2 mm, e.g. flagellata, amoeba) to megafauna (> 20 mm, e.g. earthworms, moles) (Scheffer-Schachtschabel, 1989). Visual evidences of soil fauna actions are soil mounds, rodent burrows, krotovinas, worm holes, insect cavities, insect nests, snail shells, worm casts and faecal pellets. Although the biomass of soil fauna is a relatively small portion of the total soil mass they are occupying, they are effective in moving material upward against gravity and the flow of water, in moving top soil and litter downward, in altering soil fabric and microtopography, in changing distribution patterns of soil minerals, organic matter and plant nutrients, in regulating processes and assemblages of materials and microorganisms and in adding biochemically processed components to the soil body (e.g. Hole, 1981). Principally, these actions result in:

1. Primary (direct) effects:
a) Transport of soil components in the downward and upward directions
b) Physical and biochemical conversion of soil components
2. Secondary (indirect) effects:
a) Facilitation of transport processes through faunal voids and macropores
b) Promotion of plant physiological and microbiological processes.

In view of a contamination of organic (live or dead) or mineral soil components, it is necessary to consider amounts and distances of the faunal transport of matter on one hand, e.g. for the purpose of building refuges or of storing feed, which is essentially a physical translocation within the soil profile sometimes connected with a mixing with excreta, and amounts and degree of the physical and biochemical modification of soil components via ingestion and excretion on the other hand, which may also include accumulation of radionuclides in the animal biomass.

3. Quantification Of Soil Fauna Actions and Modeling

Quantitative information on faunal transport and digestion rates is scarce, and the conditions of the investigations are not always clearly stated. One of the most effective soil animal seems to be the earthworm capable of moving soil components of up to 260 kg m^{-2} a^{-1} (Scheffer-Schachtschabel, 1989). Using a more detailed data set on earthworm actions published by Bouché (1981), Mueller-Lemans and Van Dorp (1996) calculated a turnover time of the top 25-cm layer of 5-20 years including a total worm cast deposit on the surface of 6 kg m^{-2} a^{-1}. By modeling the moving and mixing of 2 kg m^{-2} a^{-1} between the top layer and depths down to 1 m, they come to the conclusion that for medium and strongly sorbed radionuclides,

transport in the solid phase by earthworms becomes more important than transport in the activity liquid phase.
Recently, Taeschner and Bunnenberg have presented a transport model for earthworm actions, which is also based on Bouché's data set with the additional assumption of an exponential earthworm "vitality" function with depth characterising the efficiencies of all transport and mixing processes (see Bunnenberg, 1998). Their model yields time curves of the relative activity concentrations in the top 10-cm layer for the case of radionuclides sorbed to mineral or organic soil components. Comparison with the effect of pure migration shows that only in the case of weakly sorbed radionuclides, like Tc, Sr, I, the activity decrease is faster than that resulting from bioturbation (Fig.1).

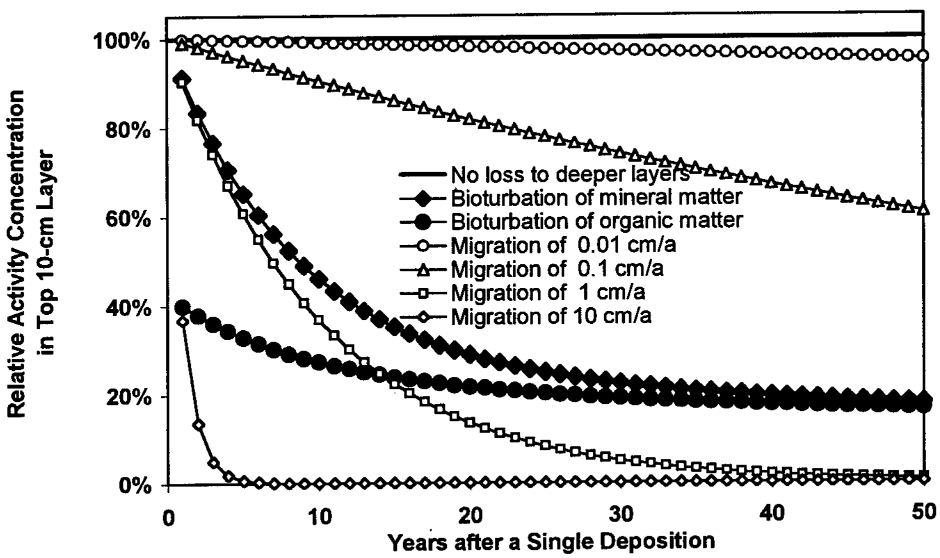

Fig.1: Time courses of the relative activity concentrations of the top 10-cm soil layer after a single deposition as a result of bioturbation of organic or mineral soil components compared to that of physico-chemical migration of radionuclides at different migration rates.

4. Conclusions

From the few data existing on soil fauna actions it can be concluded that bioturbation is at least as effective as migration in translocating radionuclides in the soil profile. This can also be visualised by a simple picture: Considering only the upward transport of uncontaminated soil, it can easily be estimated that each kilogram of soil excavated and deposited onto the surface per square meter and year by soil animals effectively induces a downward movement of the activity deposit by about one millimeter per year.

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THE ROLE OF MICROBIAL ACTIVITIES IN THE EXCHANGES OF RADIONUCLIDES BETWEEN THE SEDIMENTS AND THE WATER COLUMN

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1. Introduction

Radionuclides (Co, Cs, Sr,..) like other elements are located in biotic and abiotic compartments of the rivers. It was proved that their exchanges between the sediments and the water are mediated to some extent by microbial activities (Hambuckers et al. 1993). Microorganisms influence these exchanges by several ways e.g., they are able to sequester the elements in their cells and also to induce their inactivation as insoluble forms by immobilisation, biosorption, bioaccumulation and insolubilisation (Hambuckers and Remacle 1996). On the other hand microbial ammonification of the organic matter produces NH_4 which competes with some radionuclides such as Cs for the adsorption sites of clay (Comans et al. 1989). Bacteria specially interfer in the mineral cycling in the sediments where the bacterial density on the particulate matter can be as high as 100,000 to 600,000 cells per mm^2 (Palmateer et al. 1993). The microorganisms are therefore one of the path for the transfers of elements to the higher levels of the trophic web through the microbial loop (Overbeck and Chrost 1990). In batch experiments, it was proved that the importance and the direction of the transfers of 11 trace metals (among them Cs and Co) between the sediments and the water were controlled by microbial biomass and by microbial activities which are able to alter the physico-chemical characteristics specially pH and Eh (Hambuckers et al. 1997). However these assays were performed in closed systems. It was therefore interesting to carry out experiments in open microcosms which better mimic the conditions occurring in the rivers particularly because the sediments are continuously washed by the water flow.

2. Materials and Methods

Experiments were performed in open systems consisting of chemostats containing sediments sampled in the river Meuse in order to conduct the incubations with the "*in situ*" microbial community. After treatment with ^{134}Cs the activity of the sediment averaged 32,000 $Bq.g^{-1}$ sediment d.w. at the beginning of the assay. The non-sterile sediments were suspended in 500 ml of sterile water of the river Meuse in the chemostat. The dilution rate was chosen so that 24% of the Meuse water of the chemostat was renewed every day. The aeration was stopped 1.5 hour before sampling the water in order to prevent loss of sediments from the chemostat. The radioactivity was then measured. The microbial activity was controlled by two factors, temperature and supply of organic nitrogen, at three levels in an experimental design of 9 assays (2 factors at 3 levels). The temperatures of incubation were 4, 15, 26 °C. The concentrations of organic nitrogen in the feeding flux were 0, 100, 200 mg. L^{-1}. In all the assays, the organic carbon concentration was maintained at 400 mg. L^{-1}. The microbial activity was assessed by the ATP content in the sediments at the end of the experiments.

3. Results and Discussion

The activity budget was drawn at the end of the experiments and showed that the percentage of recovery was between –7 and + 14 % of the activity at time 0. When examining the flux of radioactivity at the output of the chemostats, two contrasting situations were observed. In the first one, the steady state was reached after approximately 2 days as shown by the unchanging flux of radioactivity. In the other one the flux of radioactivity dropped more or less sharply and reached the steady state after approximately 9 days. The radioactivity of the sediments was lowered between 12 and 41 % at the end of the assays. The output of ^{134}Cs was dependent on the conditions of incubation. The daily transfer amounted to 300-500 $Bq.g^{-1}$ of $sediment.day^{-1}$ at 4°C and no supply of organic N while it equalled 70-80 Bq. g^{-1} of $sediment.day^{-1}$ at 26°C with a supply of 200 mg. L^{-1} of organic N. It could be related to the activity of the microbial population as shown by the content of ATP in the sediments at the end of the incubation period. When incubated at 4°C and no supply of organic N, the ATP content was 6.5 lower than in the incubations at 26°C with a supply of 200 mg. L^{-1} of organic N. By taking into account the output of radioactivity, it could be theoretically assessed that around 100-450 days should be needed to completely leach the radioactivity out off the sediments depending on the microbial growth. In fact another factor has to be taken into account in this calculation because of the affinity of the clay particles for Cs (Thiry et al. 1997). It was also observed that the outlet flux contained a small amount of fine particles which are removed from the chemostats. It accounted at a maximum for 0.35% of the sediments. It seems that this loss is dependent on the microbial activities because 2.2 times more fine particles escaped the chemostats incubated at 4°C and no supply of organic N.

4. Conclusions

These preliminary results confirm the conclusions of the batch experiments (Hambuckers et al. 1997). The microorganisms could efficiently hinder the leaching of radionuclides from the sediments depending on the growth conditions. Moreover, it is possible to quantify the impact of microorganisms on the flux of radionuclides trapped in the sediments. Further researche is needed in order to understand, in rivers, the behaviour of radionuclides in the presence of microorganisms *e.g.*: the interactions between the microorganisms and the particles, the role of the clay particles, the fate of radionuclides in anaerobic conditions.

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THE ROLE OF MICROBIOTA IN THE BEHAVIOUR OF RADIONUCLIDES IN SEMI-NATURAL ECOSYSTEMS

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1. Introduction

Soil biological and plant physiological studies have shown that soil microfauna and microflora, collectively called 'soil microbiota', play a key role in most biogeochemical proces-ses in soils, like decomposition of organic material and modifications of the mobility and plant availability of elements partially performed jointly with other soil organisms. Consequently, soil microbiota deserve special attention when investigating the fate of radionuclides after deposition onto soil surfaces. This is of special importance in the case of semi-natural ecosystems, where there is a much higher level of microbial diversity and activity compared to agricultural systems because of a higher supply of organic matter and the absence of frequent disturbances resulting from agricultural management practices.

2. Observations and estimations on the effects of microbiota

There is a lot of evidence that in semi-natural environments radionuclides are found in the top soil layer for long periods of time after deposition. This is in agreement with earlier investigation into some other elements. It was estimated that microflora immobilize more than half of the mobile forms of Fe, Sr, Ca within the litter layer (Kovalsky, 1974). On the other hand, it was predicted that only 0.25% of the $^{239/240}Pu$ in an ecosystem resides in forest biota (Garten et al., 1978). Krivolutskij and Pokarzhevskij (1986) noted that the migration of soluble compounds due to non-biological factors is one order of magnitude lower than that associated with biological processes. In this context, studies on the role of soil fauna as regulators of decomposition and mineralization of nutrients, on interactions of soil fauna and micorrhizal fungi and on relationships between soil fauna and microflora are of great value (e.g. Crossley, 1984).

Soil biota can be considered a highly selective temporary 'sink' for a number of pollutants, such as some heavy metals and radionuclides. In this way, those substances are introduced into the multi-level food-webs (Pokarzhevskij, 1988) and, as a result, they are prevented from participation in the physico-chemical migration processes. Microflora strongly contribute to the radiocesium immobilization in forest soils (e.g., Bruckmann & Wolters, 1994), and up to 60% of the radiocaesium in forest biota is found in the fungi-mycelium association (Shcheglov, 1998).

Microorganisms also play a key role in the transformation of organic matter, in the formation of various simple salts from soil mineral and organic components, and they participate in the destruction and creation of *de novo* soil minerals. The mechanisms of soil mineral destruction are based on the actions of microbial oxidation-reduction enzymes as well as of various slime and acid products. Some of the organic compounds produced by microorganisms may chelate the mineral elements, creating complex compounds and favouring their migration (Fe, Mn, Al, Sr, possibly Pu, Am). Fungi are actively involved in the mineralization and humification of organic matter. They produce various acidic compounds, like citric, acetic and some other acids. This fungial ability leads to active mineral destruction. It can cause increases of the bioavailability of some radionuclides like those of Cs, which exhibits a special mechanism of element-mineral interaction. As many fungi species live in symbiosis with higher plants, they can provide them with radionuclides along with the nutrient elements. As a result of that, much greater soil surfaces are explored by the 'fungi-plant' system.

Horizontal redistribution of radionuclides due to microbiota activity could be important on the micro- and mesoscale. There is an interdependence between tree overstory and the development of understory (Anderson et al., 1969; Lieberman et al., 1989; Yastrebov, 1996), and this should be considered with respect to the fate of intercepted radionuclides. As microbiota communities are quite specific for the type of forest overstory and understory, this specificity also affects the further behaviour of radionuclides. Superimposing the two horizontal patterns of radionuclide deposit and microbiota activity may lead either to a more homogeneous distribution of radionuclides or to the opposite effect. Besides spatial variations there are also seasonal dependencies of radionuclide redistribution due to soil microbiota action. It was found that many types of forest stands showed distinct, regular and often very pronounced seasonal rhythms in radiocaesium activity and, at least for one species of fern, it was demonstrated that there is a direct correlation between the seasonal development of endomycorrhiza and the temporal patterns of radiocontamination (Nimis, 1997).

3. Conclusions

It can be concluded that because of the appreciable percentage of radionuclides at least temporarily incorporated into soil biomass, the biotic dynamics may be responsible for the large variations of migration and root uptake with time and location observed within an ecosystem. Nimis (1997) concluded that 'disregarding horizontal and vertical radioactivity patterns might result in such a high data variability that any generalization, not to speak of modelling, could be severely hampered'.

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POSSIBLE EFFECTS OF ROOT ACTION IN THE RHIZOSPHERE ON THE ADSORPTION OF TRACE AMOUNTS OF NICKEL BY VARIOUS SOILS

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1. Introduction

Adsorption is an important factor in the mobility and bioavailability of trace metals and radionuclides, including nickel. The speciation and availability of nickel have been reviewed by Uren (1992). Adsorption depends on the nature of the soil, in particular its mineral composition, but also on parameters which may vary with time, such as pH, salt concentration and the concentration in organic ligands. There is increasing awareness that the properties and composition of soil rhizosphere, the zone modified by the action of roots and associated biota, may differ considerably from the bulk soil. It is well established that such biological action may modify the availability of nutrients, such as potassium, phosphorus and iron (HINSINGER, 1998). However the consequences for mineral pollutants has received little attention. Our aim is to investigate the effect of changing pH or the addition of organic compounds on the adsorption of trace amounts or radionickel, ^{63}Ni on various soils. The organic compounds are typical of root exudates ; citrate, a protein (bovine serum albumin, BSA) and a strongly chelating complex, EDTA.

2. Materials and Methods

Surface soils with differing characteristics were sampled in three locations in France. The adsorption of nickel was measured in dilute suspension (1g/20 ml) shaken end-over-end under controlled conditions of time, temperature and ionic strength. The radioisotope, ^{63}Ni, including carrier, was obtained from Amersham International. Soil and solution were separated at the end of the adsorption period by centrifugation and the activity of ^{63}Ni remaining in solution measured by liquid scintillation. Adsorption of nickel was calculated from the loss of activity from solution. The data are presented as the distribution coefficient, K_d ($dm^3\ kg^{-1}$), defined as the ratio of concentrations in the adsorbed phase, and in solution.

The initial composition of the electrolyte solution was varied to mimic some changes in soil composition, in particular those due to biological activity in the rhizosphere. Solutions contained 0.01 M $CaCl_2$ and either 1 or 3 mM HCl or NaOH, or citrate, EDTA or BSA.

3. Results and Discussion

The role of pH in determining the adsorption of nickel for a given soil is illustrated in Figure 1. For each series the third point represents standard conditions with no addition of acid or alkali. The soils differed in their pH buffering capacity, therefore the change in pH for a constant addition of acid or alkali varied greatly. The response to pH changes is variable. For two soils maximum, or constant, adsorption was observed around the native soil pH, for the other adsorption increased continuously with increasing pH.

Figure 2 shows the effect of the addition of organic ligands on the K_d, expressed with respect to that measured under standard conditions. The presence of organic ligands decreased the adsorption of nickel. EDTA almost completely inhibited adsorption. All soils show similar trends. The dominant effect of the ligands is certainly the formation of complexes with a lesser affinity to the soil surface than the free aquo-nickel cation. However, the protein, BSA, probably coats the surface, thereby changing the surface adsorption properties. Similar effects have been observed for Cs which does not form stable complexes in solution (DUMAT and STAUNTON (1999).

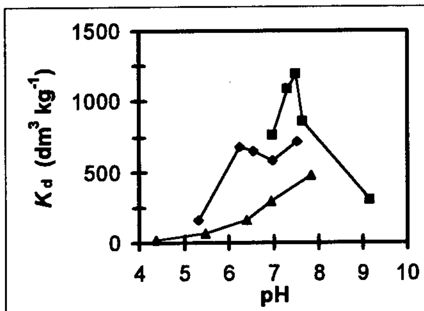

Figure 1. Effect of pH changes on the K_d of the soils

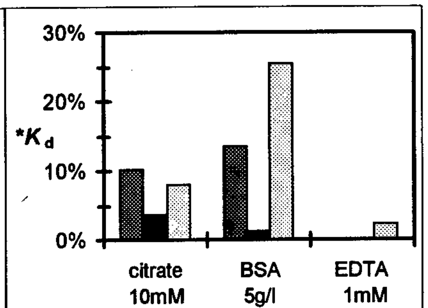

Figure 2. Reduction of K_d by organic ligands for each soil

4. Conclusions

Our findings illustrate that measurements on homogenised bulk soil should be interpreted with caution. The adsorption of nickel, and hence its mobility in soil and its availability to plants and other soil biota, may be subject to considerable variability due to the heterogeneity of soil composition. In particular, the secretion of organic anions, and the modification of soil pH by roots and fungi in the rhizosphere may considerably decrease nickel adsorption by soil.

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A CACL$_2$ 0.01 *M* SOIL EXTRACT PREDICTS THE SOIL SOLUTION COMPOSITION AND PLANT AVAILABILITY OF ^{137}CS.

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1. Introduction

Predicting food chain contamination in a ^{137}Cs contaminated area remains difficult. The collected soil to plant transfer factors show a high variability (UIR, 1989). Predictions based on soil properties have limited success (Eriksson and Rosén, 1991). Recently it was shown that the K concentration and ^{137}Cs activity concentration in soil solution predict plant availability of ^{137}Cs in soils (Absalom et al., submitted; Smolders et al., 1997). But sampling soil solutions is labour intensive and the ^{137}Cs activity in millilitre soil solution samples is below the detection limit. In this paper we propose a method to estimate ^{137}Cs and K in soil solution from a $CaCl_2$ 0.01 *M* soil extract. This way, much larger solution volumes can be extracted, avoiding the detection limit problem. The ^{137}Cs and K concentrations in the extract are compared with the in situ soil solution composition of soils that are highly contaminated with aged ^{137}Cs.

2. Materials and Methods

The top layers of ten soils were sampled, characterised, contaminated with ^{137}Cs in 1996 as described in Smolders et al. (1997) and stored moist at room temperature for two years. Soil types are listed in table 1. The ^{137}Cs activity concentration (AC) in soil ranged between 64 and 2600 kBq kg^{-1}. The soils were moistened to the values listed in table 1 and equilibrated for 5 days. The soil solution was sampled with Rhizon Soil Moisture Samplers (RMS, Rhizosphere Research Products, Wageningen, The Netherlands). After soil solution sampling, the soils were rewetted to the initial moisture content and equilibrated for one day after which 75 g of soil was mixed with 75 mL $CaCl_2$ 10^{-2} *M* in a 250 mL container. After 24 h end-over-end shaking, suspensions were centrifuged and filtered over a 0.45 µm filter. The ^{137}Cs AC's in the soil solution and in the Ca-extract were determined by γ-spectrometry (20 ml, Minaxi, 5530 auto-γ) and the K concentrations in the soil solution and in the extract were determined by atomic absorpion spectrophotometry (AAS).

3. Results and Discussion

Potassium concentrations and ^{137}Cs AC's measured in the soil solution and in the extract are listed in table 1. The K concentrations and ^{137}Cs AC's in the soil extract are lower than the respective concentrations in the soil solutions. Potassium is the major competing ion in ^{137}Cs sorption. A decrease of the K concentration upon diluting the soil solution results in a decrease of ^{137}Cs AC. This is illustrated by almost equal ^{137}Cs/K ratio's in soil solutions as in the extract (details not shown). For the sandy soils, and for the loamy and clayey soils, an average value of the K in soil solution/K in extract ratio was calculated. The K ratio of sandy soils (3.18 ± 0.49; n = 8) was clearly distinct (p= 0.05) from the K ratio of clayey and loamy soils (1.90 ± 0.17; n = 12). This can be explained by differences in the buffering power of sandy soils compared to clayey or loamy soils. The K ratios of the sandy and clayey soils were used to predict the ^{137}Cs AC in soil solution from the ^{137}Cs AC's measured in the extract. These results, combined with

^{137}Cs AC's in the soil, allowed us to predict in situ soil-soil solution distribution coefficients within a factor 1.4 (table 1).

Table 1: Soil type and moisture content (MC) of the soils. Potassium concentrations and ^{137}Cs activity concentrations (AC) in the soil solution and the 1:1 Ca-extract. The ^{137}Cs and K concentrations in soil solution are related to plant availability of ^{137}Cs (Smolders et al., 1997). $K_d^{in\ situ}$ is the soil-soil solution distribution coefficient. The predicted K_D is based on ^{137}Cs concentrations in the extract divided by 3.18 for sandy soils and 1.90 for loamy and clayey soils to account for lack of the K buffer power of the soil (see text).

			Soil solution		1:1 $CaCl_2$ 0.01 *M*-extract			
Soil	Soil type[a]	MC[b] mL g^{-1}	K conc. mM	^{137}Cs AC Bq L^{-1}	K conc. mM	^{137}Cs AC Bq L^{-1}	$K_d^{in\ situ}$ L kg^{-1}	K_D^{pred} L kg^{-1}
15	S	0.26	8.34 (1.00)	448 (3.2)	2.56 (1.41)	158 (17.8)	142	129
19	SC	0.36	1.23 (1.47)	102 (21.5)	0.68 (1.67)	53 (0.1)	26062	26003
21	C	0.31	1.83 (0.15)	170 (30.5)	1.04 (3.29)	104 (13.5)	13987	11575
24	CS	0.27	5.24 (0.62)	478 (3.7)	2.09 (0.43)	186 (2.5)	2539	2044
30	LS	0.23	7.32 (0.59)	986 (7.7)	2.31 (1.17)	258 (1.4)	732	878
35	L	0.26	2.87 (0.19)	368 (6.5)	1.28 (0.14)	143 (1.5)	5027	3770
53	S	0.22	9.70 (0.45)	10088 (0.6)	2.55 (0.64)	2537 (0.2)	15	18
69	C	0.36	2.25 (0.84)	196 (0.05)	1.16 (1.48)	132 (4.4)	5034	3944
80	C	0.32	0.60 (3.52)	584 (7.4)	0.32 (1.40)	299 (0.8)	2925	3000
84	C	0.53	1.07 (0.68)	68 (28.8)	0.60 (1.66)	33 (12.0)	4035	4206

[a] S: sand, L: loam, C: clay, CS: clayey sand, LS: loamy sand, SC: sandy clay; [b] Moisture content of the soil samples. All data are means and coefficients of variation (%; in brackets) of two replicates.

4. Conclusions

A single step extraction procedure with $CaCl_2$ 0.01 *M* allowed us to predict soil-soil solution distribution coefficients of ^{137}Cs within a factor 1.4. When working with large volumes (1 kg soil, 1 L $CaCl_2$ solution), the proposed method is practical to use in field conditions: the soil volume is easy to collect and the large solution volume will ensure an adequate ^{137}Cs AC to be measured.

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MOBILITY OF ^{137}CS AND ^{90}SR RELATED TO SPECIATION STUDIES IN CONTAMINATED SOILS OF THE CHERNOBYL AREA

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1. Introduction

Among the parameters likely to affect the radionuclides migration in soils and so their availability for the environment, the nature of the fallout and the physico-chemical characteristics of soil type are of main interest. The deposits issued from the Chernobyl accident have borne two main physico-chemical forms (condensed forms of aerosols and fuel particles, « F.P. »), whose behaviour in terms of potential mobility of radionuclides has been different during the course of time [IVANOV et al. (1997)]. Because of their serious radiological hazard, we focused our study on ^{137}Cs and ^{90}Sr isotopes, widely released into the atmosphere during the accident. In order to improve the knowledge of their mobility in different soil layers, we chose an approach based on a selective chemical extraction protocol, and the first results are presented in this paper.

2. Materials and Methods

According to the considerations described above, several sites of different situations in Ukraine, Belorussia and Russia were sampled in 1996. The soils investigated were representative of the region (soddy-podzolic, sandy, peaty and gley soils) and showed different contamination forms and levels (Table 1). After the determination of ^{137}Cs and ^{90}Sr vertical distributions by gamma spectrometry and liquid scintillation, we evaluated on the soils the desorption potential of several chemical reagents through a classical sequential extraction scheme adapted from TESSIER et al. (1979) : - soluble fraction (H_2O), - exchangeable fraction (NH_4OAc, NH_4Cl, $CsCl$, $MgCl_2$), - reducible fraction (NH_2OH-HCl in CH_3COOH), - oxydable fraction ($K_4P_2O_7$, H_2O_2 + NH_4OAc) and finally - residual fraction (mineralization.). After kinetic desorption studies and complementary measurements on the leachates, a compromise between reagent selectivities and performances will be chosen, which will allow us to design a single protocol especially adapted to the studied soils.

3. Results and Discussion

In agreement with its slow migration in soils [IVANOV et al. (1997)], ^{137}Cs is on a non soluble state (barely 1% of the total activity desorbed), although we observed high desorption rates, up to 45% for the « Rad. » site while using a 1M NH_4OAc extraction solution. This confirms the great efficiency of NH_4^+ to desorb caesium fixed on specific sorption sites. The only site with a behaviour different from the others is « Ch.T », with less than 1% of the total ^{137}Cs desorbed. This soil presents the particularity of containing a high amount of undestructed fuel particles that contains radiocaesium. The presence of these F.P., not leachable by a neutral salt [KASHPAROV et al. (1997)], could be the principal reason of the low-desorption of ^{137}Cs in this

fraction. Moreover, the exchangeable fraction of radiocaesium increases with depth (Figure 1), where F.P. have not migrated [ECP2 (1991-1995)].

4. Conclusions

The first results about the ^{137}Cs mobility allow us to correlate the low migration rate of this nuclide with the presence of clay minerals in soils, even in a few percents, and the existence of undestructed fuel particles. The part of caesium that migrates could be assimilated to the soluble and exchangeable fractions being available in presence of a saline solution (competing cations). But the mobility studies are not only devoted to these first fractions : the following fractions of our protocol will allow us to ascertain the role played by the Fe and Mn sesquioxides and the organic matter on ^{137}Cs and ^{90}Sr retention.

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Table 1 : Brief description of the sites

Site	soil type	type of deposit	surface massic activities (Bq/g)		clay content (<1%)	organic matter %	
			137Cs	**90Sr**		**0-5 cm**	**6-9 cm**
Ch.P	podzol	low oxidised F.P.	170	400	illite, smectite	0.8	0.8
Ch.T	peat	low oxidised F.P.	1400	1700	kaolinite	84	90
Pry.	sand	high oxidised F.P.	250	100	kaolinite, smectite	0.2	0.2
Rad.	peat	F.P., condensed forms	560	160	kaolinite	30	34
Zym.	gley	high oxidised F.P.	140	80	chlorite, smectite	11	4

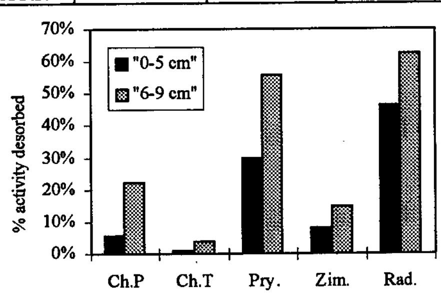

Figure 1 : ^{137}Cs exchangeable fraction at different depths1M NH_4OAc solution, 24h contact

FLUXES OF ^{137}CS, ^{60}CO AND ^{226}RA IN THE SOIL-WATER-PLANT SYSTEM AS DETERMINED BY LYSIMETER EXPERIMENTS

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1. Introduction

The results of seven years of lysimeter experiments to determine radionuclide leaching from cultivated soils are presented and the uptake of ^{60}Co, ^{137}Cs and ^{226}Ra into agricultural crops (endive, mustard, potato, maize, Faba beans, ryegrass, wheat, sugar beet) is described.

2. Materials and Methods

The lysimeter consists of twelve monolithic soil profiles (four soil types: Eutric Cambisol, Dystric Cambisol, Dystric Cambisol on cristalline rock, Dystric Gleysol and three replicates) and is located in Seibersdorf/Austria, a region with a pannonian climate. Leachate water collection and gammaspectrometric analyses allowed derivation of radionuclide losses with seepage water. For details see Gerzabek et al. (1998).

3. Results and Discussion

The highest losses were recorded for ^{60}Co, a similar magnitude was estimated for ^{226}Ra, ^{137}Cs exhibited the lowest losses. There was no indication for a breakthrough of radionuclides, but for constant small losses.

Besides soil-to-plant transfer factors soil-plant fluxes were calculated, taking into account biomass production and growth time according to the method suggested by the UIR working group on radionuclide fluxes (applying a single exponential term for flux calculation, Mitchell, person. comm.). As an example for flux determinations the data for maize are presented here. The calculated values for maize straw vary among different soil types; fluxes to grains are always lower than those to vegetative plant parts. For the three radionuclides we found the following average fluxes (s^{-1}):

^{137}Cs: straw: from 3.40×10^{-12} to 1.47×10^{-10} (mean: $7.31 \pm 7.07 \times 10^{-11}$); grain: $1.18 \pm 1.29 \times 10^{-11}$; ^{60}Co: straw: from 2.00×10^{-12} to 3.11×10^{-12} (mean: $2.44 \pm 7.49 \times 10^{-13}$); grain: $2.76 \pm 1.40 \times 10^{-13}$; ^{226}Ra: straw: from 8.17×10^{-12} to 3.51×10^{-11} (mean: $2.35 \pm 1.20 \times 10^{-11}$); grain: $3.76 \pm 3.40 \times 10^{-13}$.

It can be concluded that the radionuclide amount extracted from soil due to harvest of agricultural crops under normal conditions is small. Despite the high rate of biomass production

of 2.37 ± 0.30 kg maize straw and 1.16 ± 0.16 kg maize grains per m^2 (arithmetic mean ± standard deviation) the amount of radionuclides extracted by a single harvest represents on average 0.105%, 0.003% and 0.031% of the soil inventories for ^{137}Cs, ^{60}Co and ^{226}Ra. Nevertheless, plant extraction exceeds the losses due to radionuclide leaching in soil solution considerably. Approximated leaching losses per year amounted to 0.4×10^{-4}%, 3.9×10^{-4}% and 4.3×10^{-4}% for ^{137}Cs, ^{60}Co and ^{226}Ra.

4. Conclusions

The losses of soil inventory by maize harvest are more than three orders of magnitude (2400 x) higher than leaching losses in the case of ^{137}Cs, approximately two orders of magnitude higher (70 x) for ^{226}Ra and only one order of magnitude higher (9 x) for ^{60}Co. However, both in the case of ^{137}Cs and ^{60}Co the most important loss is the physical decay due to the relatively short half-life of these nuclides. Only for ^{226}Ra does the plant uptake and removal result in higher losses than the physical decay (Figure 1).

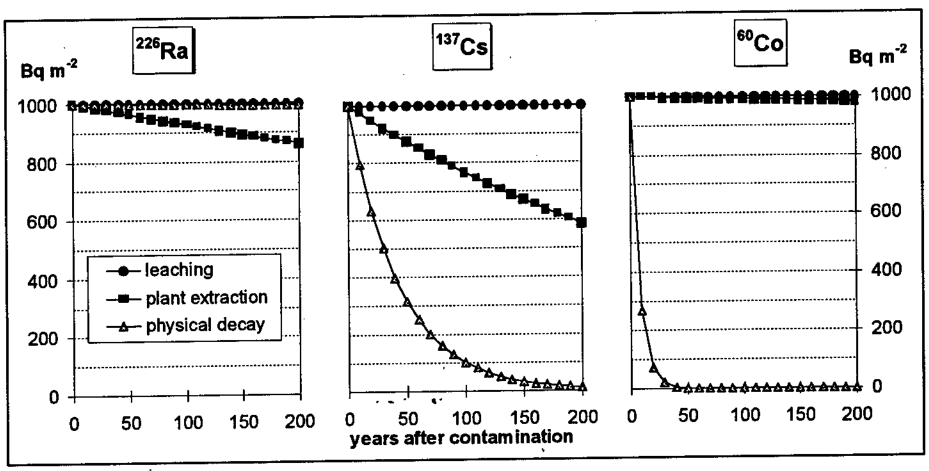

Fig. 1: Estimated radionuclide losses from soil via plant harvest, soil leaching and physical decay over a period of 200 years

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RADIOCAESIUM AND POTASSIUM DYNAMICS IN A WILLOW SHORT ROTATION COPPICE STAND

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1. Introduction

Large areas in Europe were contaminated with long-living radionuclides after the explosion of the Chernobyl nuclear power plant. Among the many countermeasures studied to mitigate the radiological consequences of the accident (e.g. Renaud and Maubert, 1997), changing the land use from food crops to non-edible industrial crops (e.g. flax, wood) stands out. However, studies about radionuclide behaviour in those industrial crops are limited.
Present paper reports the results of a detailed caesium cycling study carried out in a willow Short Rotation Coppice (SRC). A comparison was made between the distribution of radiocaesium and potassium over the main compartments of the SRC willow stand.

2. Materials and Methods

A willow (*Salix viminalis* L., var. Orm) SRC was established in 1996 on a sandy soil (experimental plots were 2 by 2 m^2, four replicates) of which the upper 25 cm were artificially contaminated with ^{134}Cs (10 MBq m^{-2}). More information about the soil characteristics is given by Gommers *et al.* (1998). The study was conducted during the second year of the culture after being established and after the stand was cut back at the end of the first growing season. Shoots (wood) and leaves were sampled five times during the growing season, roots and initial cuttings of 3 plants per plot only at the end. Litter was regularly collected by litter traps. From August until December, rain-water was collected in gutters suspended below the plant canopy to measure the elements that were leached from the foliage. Oven dry plant material (105 °C) and water samples were analysed for ^{134}Cs (Minaxi, 5530 auto γ-counter) and K content (Atomic Absorption Spectrophotometry). All activities reported were corrected for radioactive decay.

3. Results and Discussion

Biomass production was determined and was very fast before August, but leveled off afterwards. ^{134}Cs concentrations in wood and leaves increased in the beginning of the growing season, while K concentrations decreased (data not shown). This difference between K and ^{134}Cs may be related to an increasing availability of ^{134}Cs in the soil early in the beginning of the growing season, while K availability is possibly reduced due to an important supply to the plants. A decrease in K concentrations in the soil solution is known to result in an increased ^{134}Cs uptake by the plants (Smolders *et al.*, 1996). Moreover, the release of radiocaesium from the previous year litter contributed probably to the enrichment of the available Cs pool in the soil. Both, the biomass production rates and the evolution in ^{134}Cs and K concentrations in different plant parts determine the ^{134}Cs and K contents in plants (figure 1). The combination of a high biomass production rate and increasing ^{134}Cs concentrations in the plants resulted in a higher relative uptake rate for ^{134}Cs than for K between April and August. In autumn, the retranslocation

towards the below-ground plant parts is similar for K and ^{134}Cs (12.2 % and 14.6 % of the maximal content is translocated, respectively). The amount leached by rainwater accounted for 8.7 % for K and 4.7 % for ^{134}Cs. 51.6 % of the maximal Cs content in above-ground plant parts was returned to the soil in autumn by litter fall. This was 42.0 % for K.

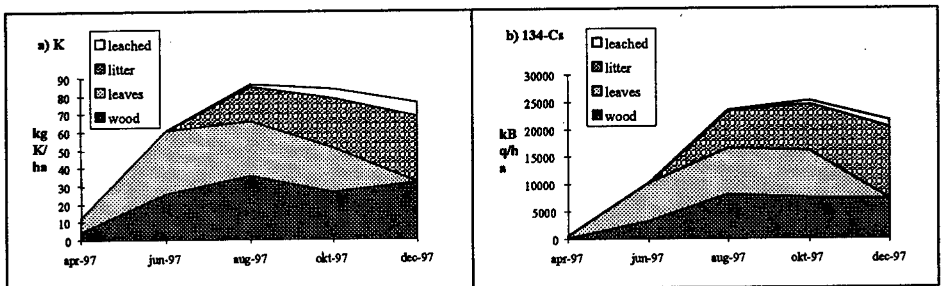

Figure 1: Evolution of potassium (a) and radiocaesium (b) contents in a willow SRC stand

Potassium in the wood represents a larger fraction than radiocaesium (37.2 % and 28.0 % of the maximal contents for K and ^{134}Cs, respectively). Radiocaesium concentration in the wood remained low (615 $\pm$ 102 Bq kg^{-1}). The below-ground plant parts (roots and original cuttings), accumulate relatively less potassium than radiocaesium. In roots and cuttings, 11 times less K was found than in above-ground plant parts, while this is only 2 times less for ^{134}Cs. Radiocaesium concentrations in roots reached up to 5493 $\pm$ 899 Bq kg^{-1}.

The total amount of radiocaesium accumulated by the plants at the end of the growing season (below- and above-ground plant parts) accounted for only 0.037 % of total radiocaesium in the soil.

4. Conclusions

Only a small fraction of the radiocaesium in the willow SRC ecosystem is taken up by the plants. A major fraction of the plant ^{134}Cs and K contents is returned in autumn by litter fall or is leached by rain-water. A large proportion also remains in or is returned to the below-ground plant parts. The fraction of radiocaesium accumulated in the wood remains low.

The distribution of K and ^{134}Cs between the above-ground plant components shows a similar pattern, but the dynamics over the growing season are different. Radiocaesium behaviour in a willow SRC can, therefore, not be simply related to what is known from potassium.

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MODELING THE SOIL-TO- PLANT CONCENTRATION RATIO FOR THE RADIONUCLIDE ^{63}NI

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1. Introduction

In safety assessment of nuclear waste disposal, the soil is one of the most significant compartment of the biosphere that controls the transfer of some radionuclides through the food chain. Hence, the accurate modeling of the soil-plant transfer of radionuclides should be a basic objective for assessment. In the case of radioelements with naturally occurring stable isotopes, isotopic exchange with the stable isotopes is the prevailing mechanism that controls the fate of the radionuclide (ECHEVARRIA, 1996). Using this approach, isotopic exchange kinetics (IEK) method was shown to be suitable and fast to describe the phytoavailability of the radionuclide ^{63}Ni in soils. Consequently, soil-to-plant concentration ratio of ^{63}Ni (CR^{63}_{Ni}, *i.e.* $^{63}Ni_{plant}/^{63}Ni_{soil}$) should be inversely proportional to the isotopically exchangeable stable Nickel ($E_{Ni}(t)$) in the soil (ECHEVARRIA, 1996).

This study was undertaken to establish the relationship between CR^{63}_{Ni} and $E_{Ni}(t)$, and to assess the possible influence of other plant parameters (e.g. dry matter yield) on CR^{63}_{Ni}. Thus, two plants were grown, a root and a leaf crop (radish and lettuce), on three different soils, and correlation coefficients between CR^{63}_{Ni}, $E_{Ni}(t)$ and other relevant parameters were compared.

2. Materials and Methods

Samples of the A_p horizon of three different soils were collected from a brown rendzina (soil 1), an ancient fersiallitic soil (soil 2) and a gleyic leached brown soil (soil 3). Soils displayed a large range of properties and fertility level. They were air-dried and sieved through a 5 mm mesh. Total initial stable nickel content was 36, 11.7, and 9.9 mg.kg^{-1} respectively.

Isotopic exchange kinetics adapted to Ni were used to calculate the quantity of isotopically exchangeable Ni (*i.e.* $E_{Ni}(t)$) (ECHEVARRIA et al., 1998). Soils were fertilized and spiked with ^{63}Ni solution (100 kBq.kg^{-1}dry soil). Lettuce (*Lactuca sativa* L.) and radish (*Raphanus sativus* L.) were grown on the three soils. During cultivation, soils were moistened daily to 100% of the field capacity. Plants were harvested after 5 weeks, oven-dried at 80°C for 48 hours, weighted, and acid-mineralized. Radioactivity was counted and total Ni measured by AAS. ^{63}Ni concentration ratios were then calculated.

3. Results and Discussion

Isotopically exchangeable stable Ni, $E_{Ni}(t)$, is usually an estimate of Ni phytoavailability in soil. It varied from 1.6 to 2.2 mg Ni kg^{-1} soil. Uptake of ^{63}Ni by plants was found to be very different on the three soils, although, for the same period of time, $E_{Ni}(t)$ was quite similar. Dry matter yield (DM) ranged from 0.19 to 0.69 g kg^{-1} soil for radish roots, and from 0.48 to 1.9 g kg^{-1} soil for lettuce after 5 weeks (Table 1). They were always higher on soil 1 and lower on soil 2 according to their respective fertility level. Total nickel taken up by plants (Ni_p) ranged from 0.2

to 1.3 μg $Ni.kg^{-1}$soil for lettuce after 5 weeks (Table 1) and from 0.19 to 0.2 μg Ni kg^{-1} soil for radish.

Table 1 : $E_{Ni}(5w)$ $CR^{63}{}_{Ni}$ and Ni_p on soils for radish and lettuce grown for 5 weeks (mean values ± standard deviation, (n=5).

		DM	CR			Nip		
	Soil Organic N	**E_{Ni}(5 w)**	**Radish**	**Lettuce**	**Radish**	**Lettuce**	**Radish**	**Lettuce**
	($g\ kg^{-1}$)	($g\ kg^{-1}$)	($g\ kg^{-1}$ soil)	($g\ kg^{-1}$ soil)	(%)	(%)	(μg $Ni.kg^{-1}$)	(μg $Ni.kg^{-1}$)
1	3.03	1.6	0.67 (0.15)	1.9 (0.86)	0.04 (0.02)	0.04 (0.018)	0.20 (0.10)	1.30 (1.20)
2	1.13	1.7	0.19 (0.03)	0.48 (0.07)	0.04 (0.01)	0.048 (0.029)	0.19 (0.05)	0.35 (0.14)
3	1.18	2.2	0.36 (0.14)	0.56 (0.19)	0.02 (0.01)	0.016 (0.012)	0.20 (0.10)	0.20 (0.04)

$CR^{63}{}_{Ni}$ were always higher on soil 2 and Ni_p was generally higher on soil 1 (Table 1). Variability was important between both species and soils. For similar values of $E_{Ni}(t)$, $CR^{63}{}_{Ni}$ were inversely related to DM, *e.g.* soil 1 and 2, radish. Moreover, for a same yield, (*e.g.* soil 2 and 3, lettuce), $CR^{63}{}_{Ni}$ were not equal but inversely related to $E_{Ni}(5w)$. Pearson correlation and probability matrix showed that the correlation coefficient between the 4 parameters was highly significant (P = 0.000), and that correlation between DM and $CR^{63}{}_{Ni}$ was significant with a 90% confidence interval. Thus, $CR^{63}{}_{Ni}$ was related to the dry matter yield, Ni_p and $E_{Ni}(t)$ using the following model (ECHEVARRIA, 1996):

$CR^{63}{}_{Ni} = A * Ni_p / (E_{Ni}(t) * DM(t))$ (with A = constant)

$E_{Ni}(t)$ DM and Ni_p accounted for 65% of the variability for the total $CR^{63}{}_{Ni}$ population ; A = 71 ± 22 with a 95% confidence interval. The negative correlation between $CR^{63}{}_{Ni}$ and $E_{Ni}(t)$ in this model gives evidence for hypothesis that the radionuclide is diluted in the phytoavailable pool of the stable element.

4. Conclusions

Concentration ratios for the radionuclide ^{63}Ni can be estimated from the measurement of the DM and $E_{Ni}(t)$ by IEK. Further studies should focus on parameters that influence the DM (e.g. fertilization), and on the significance of the A parameter. This method can be adapted to other naturally occurring elements, and be suitable for safety assessment.

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EFFECT OF SOIL K-STATUS AND BENTONITE ADDITIONS ON RADIOCAESIUM TRANSFER

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1. Introduction

After the Chernobyl accident, the study of the fate of radiocaesium in the environment was of particular importance, given its long half-life, its widespread contamination and its behavioural similarity with K. The role of K in soil-to-plant transfer has been the subject of many studies (Nisbet, 1993) but its effect is not always unequivocal. In the present experiment, the influence of soil K-status (soil solution K, [K] or exchangeable K: Z_K) on ^{134}Cs uptake in ryegrass was studied.

2. Materials and Methods

The experimental set-up consisted in total of 13 treatments, including a control treatment (C). Moist (25%) sandy soil batches, homogeneously contaminated with ^{134}Cs were amended with K-bentonite (2, 1, 0.75, 0.5, 0.25, 0 %: A1-A6 or 1% bentonite: treatments B1-B6). Specific amounts of K and Ca nitrate were added to obtain constant [K] with varying Z_K (A-series) or varying [K] at constant Z_K (series B). Both treatments C and A6 have no bentonite added but contrary to the control A6 has extra K added. After 4 weeks' incubation, the soil batches were distributed over 7 pots and ryegrass was sown. Seven cuts were harvested and shoot dry weight and K and Cs content recorded. Through appropriate fertilisation it was attempted to keep the soil K-status constant. Soil solution composition (^{134}Cs and K concentrations) and exchangeable K was measured at the end of the 4 weeks' incubation period and after 7 cuts. Transfer factors (TF: Bq g^{-1} plant/ Bq g^{-1} soil), concentration factors (CF: Bq g^{-1} plant/ Bq ml^{-1} soil solution) and radiocaesium solid-liquid distribution coefficients (K_D: Bq g^{-1} soil/ Bq ml^{-1} soil solution) were calculated. For the discussion, the soil characteristics at the end of the experiment are related to the plant data of the 7th harvest and the initial soil properties with the plant data of the 2nd cut.

3. Results and Discussion

Analysis of soil after 4 weeks incubation systems almost confirmed the treatments. In series A soils, K concentrations varied between 6 and 10 mM while Z_K varied over a wider range (0.1 - 10 $cmol_c\ kg^{-1}$). In series B soils, nearly constant exchangeable K was achieved (0.4 - 0.57 $cmol_c\ kg^{-1}$) while [K] varied (6-19 m*M*). Notwithstanding the fertilisation, soil solution K decreased drastically (factor 3 to 150) due to luxurious K-consumption by ryegrass and, more importantly, since bentonite fixed K (see below).

Figure 1 shows a summary of soil-plant TF's for all systems studied and for the 7 cuts. The lower curve gives an estimate of all TF's for the systems with more than 0.25 % bentonite added (A1-A4 & B1-B6). Given that the total soil activity is comparable for all treatments (~400 Bq g^{-1})

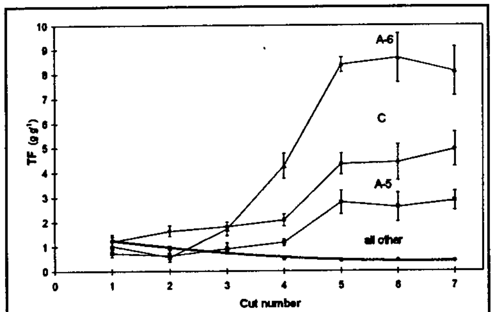

Figure 1: TFs for 7 cuts for treatments with 0 (C and A6), 0.25 (A5) or >0.25 % K-bentonite added

the evolution of plant activity concentrations is similar. It was found that ^{134}Cs uptake at the plant level was both affected by the ^{134}Cs and K concentrations in the soil solution. Considering all corresponding soil and plant data from the 2nd and 7th harvest, this relationship could be expressed as:

$$\log CF = 2.446\ (\pm 0.046) - 1.215\ (\pm 0.025) \log [K],$$

explaining 96 % of the variation. A similar equation can be deduced for the TF since

$$TF = CF/K_D.$$

The effect of Z_K on ^{134}Cs CF and TF could not be quantified as was done for [K].

Radiocaesium transfer and Z_K were negatively correlated for series A. However, for series B the correlation coefficients were insignificant.

The steady decrease in TF for treatments with more than 0.25 % bentonite is surprising. We hypothesise that this decrease reflects a built-up of a pool of highly specific Cs sorption sites following drying-wetting during plant growth. This hypothesis is supported by the fact that for bentonite amended soils, ^{134}Cs TF decreased with time, whereas an increase is observed for the treatments without bentonite. Likewise, available K-loss increased with bentonite addition (Vandenhove et al., 1998). Vandenhove and Cremers (1998) detail quantification of this effect in terms of selectivity characteristics.

4. Conclusions

Radiocaesium transfer could be reliably predicted from the K-concentration in the soil solution and the ^{134}Cs solid liquid distribution coefficient. However, no such quantitative relationship could be derived for the exchangeable K content. Bentonite in the K-form developed highly specific sorption sites upon the drying-rewetting of the soil bentonite system during plant growth and is hence a potential powerful countermeasure.

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ALTERNATIVE AGRICULTURE AND FATE OF CS-137 AND SR-90 IN THE CHERNOBYL EXCLUSION ZONE.

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1. Introduction

The presence of radionuclides in soil and water often jeopardizes ecosystem stability and poses serious risk to human health. Over 260,000 km^2 still have more than one curie per km^2 of contamination with ^{137}Cs (Shcherbak, 1996). Rational usage of these territories is one of the major challenges. Phytoremediation may provide an alternative for the contaminated arable lands (Salt, et al., 1998).

2. Materials and Methods

An experimental plot was established on the heavily contaminated soil at the North-West border of Chernobyl, Ukraine, approximately 10 km south of the ChNPP fourth reactor that was damaged in 1986. The bioaccumulation coefficient (K_b), calculated as a ratio of ^{137}Cs concentration in the plant versus ^{137}Cs concentration in the soil, was used to evaluate ^{137}Cs phytoextraction. The experimental plot was subdivided into 2 m x 2 m blocks with 1 m borders and was used to screen high biomass crops for ^{137}Cs and ^{90}Sr accumulation. Plants were seeded by hand in rows and were weeded and watered as needed. After 9 weeks, plants were harvested, air dried, and analyzed for ^{137}Cs activity. Soil and plant samples were air dried prior to analyses. The activity of ^{137}Cs in the soil and plants was determined directly by gamma-spectrometry using a HPGe detector, coupled to a multi-channel.

3. Results and Discussion

The top soil radionuclides activity did not vary significantly over time. However, meaningful heterogeneity in radionuclides horizontal distribution within the experimental plot was noticed. Concentration in the soil was for ^{137}Cs in the range from 988 Bq kg^{-1} to 1937 Bq kg^{-1}, and for ^{90}Sr from 873 Bq kg^{-1} to 2316 Bq kg^{-1}. Bioaccumulation in the above-ground plant biomass was higher for ^{90}Sr (K_b from 4.72 to 22.89) compared to ^{137}Cs (K_b from 0.06 to 4.08) (Table. 1). Overall, *Amaranthus* species proved to be good accumulators for both radionuclide tested and the best accumulators for ^{137}Cs. High levels of ^{90}Sr in *Helianthus* species in conjunction with high biomass lead to the total removal up to 28,000 Bq m^{-2}. Notable variability in accumulation of radionuclides is observed for the crop species.

Table 1. Radionuclide accumulation in plants grown in the Chernobyl Exclusion Zone.

Species	Concentration in plants (Bq kg^{-1})		Bioaccumulation coefficients		Total removal Bq m^{-2}	
	^{137}Cs	^{90}Sr	^{137}Cs	^{90}Sr	^{137}Cs	^{90}Sr
Amaranthus bicolor L.	1220	10340	0.59	14.77	417	3536
Amaranthus caudatus L.	2800	16010	2.03	13.12	1144	6543
Amaranthus cruentus L.	2350	16350	1.32	17.87	1250	8701
Amaranthus cruentus L. cv. mironivka	2030	12325	1.07	9.84	1195	5865
Amaranthus cruentus L. cv. paniculatus	984	6700	0.53	7.40	412	2806
Amaranthus hybridus L.	1300	10420	0.60	11.51	719	5763
Amaranthus retroflexus L.	2900	8850	1.50	9.83	3225	9843
Amaranthus retroflexus L. cv. anthey	1530	9090	1.07	11.81	488	2901
Amaranthus retroflexus L. cv. aureus	3610	15790	1.90	18.69	2440	10673
Amaranthus retroflexus L. cv. belozerny	2910	13490	1.41	7.87	1392	6454
Amaranthus retroflexus L. cv. Pt-95	4716	21750	4.08	22.89	2000	9222
Brassica juncea (L.) Czern.	297	22400	0.06	11.60	29	2146
Helianthus annuus L.	599	19960	0.24	11.15	319	10619
Helianthus tuberosus L.	523	17100	0.30	19.00	846	27651
Helianthus tuberosus L. x *H. annuus* L.	864	7750	0.49	8.90	1221	10951
Zea mays L.	458	7830	0.07	4.72	67	1143

4. Conclusions

Phytoremediation may be a valuable option for the soils with low level radionuclide contamination. High biomass crop plants are capable to remove substantial amount of radioactivity from the soil. Phytoremediation targeted the most bioavailable portion of radionuclides in the soil. That implies that phytoremediation may be used in crop rotation as a preceding to traditional crop growth.

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FATE OF ^{99}TC IN A SOIL-PLANT-ANIMAL SYSTEM

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1. Introduction

The fate of 99Tc in soil-plant systems has been thoroughly investigated and so has been its fate in plant-animal systems. Nevertheless, research was seldom directed to study the whole agrosystem (i.e. soil-plant-animal-system). It is generally agreed that in aerated environments, Tc is very mobile as TcO_4^-, and easily transferred to plant leaves, showing very high concentration ratios (ECHEVARRIA et al., 1997). Tc has been shown to be transferred to animal as pertechnetate or bound to plant material. Its absorption coefficient in animals based on (ingestion - feces) difference is low, especially for ruminant (GARTEN et al., 1986; VAN BRUWAENE et al., 1986; Gerber et al., 1989). During an intravenous injection in the ruminant, major part of Tc is excreted in feces (GERBER et al., 1989). The difference (ingestion - feces) also probably underestimated the real absorption of Tc by the ruminant. This work was undertaken to study the fate of ^{99}Tc in the continuum and assess the bioavailability of ^{99}Tc at two levels: in the soil for plants and in the plant for animals.

2. Materials and Methods

A soil sample from the A_p horizon of a French Calcic cambisol (silty loam, $pH_{water} = 6.4$) was used. Soils were fertilized and received Tc as NH_4TcO_4 (25kBqÝkg^{-1}). Ryegrass (*Lolium perenne* L., var. Prana, i.e. RG) was grown under three levels of N fertilization (21, 50 and 100 mg N kg^{-1}, mainly as N-NO3). All treatments were made in 5 replicates. Three cuttings were made every four weeks. At harvest, RG shoots were weighed, oven-dried (70°C for 24h) and aliquots were mineralized through acid digestion. Radioactivity in samples was counted. Total nitrogen content was determined by the Kjeldahl method (ECHEVARRIA et al., 1998).
Location of ^{99}Tc in RG was determined after separation of cell-wall constituents (NDF, ADF and ADL) according to VAN SOEST and WINE (1967). ^{99}Tc solubility in rumen was modeled by incubating $^{99}TcO_4^-$ or ^{99}Tc bound to RG in ruminal juice and counting radioactivity in solution and bacteria. Its uptake by ruminal bacteria was expressed as the percentage of introduced radioactivity. Ability of ^{99}Tc to cross digestive wall was tested *in vitro* with a diffusion test through a jejunum or a duodenum segment filled with Ringer solution or ruminal juice. Three ewes (carotide catheterized) were fed with $^{99}TcO_4^-$ or ^{99}Tc bound to RG and blood samples were taken until 48h.

3. Results and Discussion

Total uptake of ^{99}Tc decreased with increasing NO_3^- additions at the first cutting, as N was not a limiting factor yet (as shown by total N content in shoots). ^{99}Tc content in plants varied from 64% of the applied ^{99}Tc with the lowest NO_3^- addition to 31% with the highest. However, at the third cutting, cumulative ^{99}Tc uptake reached the same level for the 3 treatments (80 to 83% of applied ^{99}Tc, without any significant difference) with no further effect of N, as NO_3^- was depleted in the soil. Thus, $^{99}TcO_4^-$ uptake by plant roots was strongly inhibited by the presence of

NO_3^- ions in soil, suggesting a possible dilution of $^{99}TcO_4^-$ in the pool of soil NO_3^- (ECHEVARRIA et al., 1998).
A major part of ^{99}Tc-RG (lowest N-addition treatment) was found in the cytoplasm (58%) and in the hemicellulosic fraction (40%) of RG leaves. No significant amount was found in lignin and only 18% in cellulose. ^{99}Tc was then present in easily solubilized fractions of RG. These results were in agreement with the high speed of ^{99}Tc solubilization either in water or in ruminal juice and with results reported by GARTEN et al. (1986). Despite of this location in accessible compartments of the plant, RG-incorporated ^{99}Tc was less taken up and at a slower rate by ruminal bacteria than $^{99}TcO_4^-$. ^{99}Tc bound to RG was probably not only as $^{99}TcO_4^-$ form and this new chemical form decreased its adsorption on bacteria membranes or its absorption. ^{99}Tc was found to be unable to cross intestinal epithelium without blood circulation. There was no passive diffusion even after 6 h of incubation. But when ewes were fed, ^{99}Tc was found in blood samples, which proved its ability to migrate from intestinal tract to blood, probably by an active mechanism.

4. Conclusions

Results showed that the soil-to-plant transfer in aerated conditions under which, $^{99}TcO_4^-$ is the predominant form over the long term, is controlled by the presence of nitrate in the soil. Therefore, the N cycle in the soil might have a strong influence in ^{99}Tc transfer to crops. ^{99}Tc absorbed by the plant was mostly transferred to RG leaves (more than 90%) and should be in such conditions as readily available as $^{99}TcO_4^-$ to the next crops if it reincorporated to the soil under this form (ECHEVARRIA et al., 1997). Ruminants were able to take up ^{99}Tc in food. Plant-incorporated Tc was less mobile in ruminal system than $^{99}TcO_4^-$. The understanding of the cycle of ^{99}Tc in the whole agrosystem would be more complete if focus was made on the bioavailability of ^{99}Tc excreted in animal feces to plants and the effect on the ^{99}Tc cycle in the soil.

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VAN SOEST, P.J. and R.H. WINE (1967): Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell wall constituents. Assoc. Agric. Chem. J., 50: 50-55. soil-plant-ruminant system

INVENTORY AND HORIZONTAL VARIABILITY OF CAESIUM-137 IN RAINFOREST SOIL OF COSTA RICA

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1. Introduction

The behaviour of ^{137}Cs in forest ecosystems in Northern temperate latitudes has been studied extensively during the last years, in particular after the Chernobyl accident. Various studies about the spatial variability of fall-out nuclides have been carried out in semi-natural ecosystems, mostly in open grassland, but some also in forests; in Austria e.g. by STREBL (1996) and FEA (1998). However, very few detailed studies seem to exist about radionuclide distribution in tropical forest soils. Recent literature includes investigations in Southern India (SOMASHEKARAPPA et al., 1996) and a study from Taiwan which presents some figures about the depth distribution of radionuclides in soils from undisturbed open areas in forest land (SHU-YING LAI et al., 1996). The contamination due to global fallout from weapon tests is more or less evenly distributed longitudinally but shows a distinct latitudinal dependency which is well known on a large scale. For the investigated Costa Rican location, a ^{137}Cs inventory of around 600 Bq/m² (decay corrected 1 Jan 1996) can be estimated from UNSCEAR data. On a local scale, however, the contamination is strongly correlated to rainfall, because "wash-out" is the most efficient way of deposition. A rainforest rehabilitation project in Southern Costa Rica sponsored by Austrian authorities gave the opportunity to carry out an investigation on this subject.

2. Materials and Methods

The investigation area is located in a hilly, high rainfall region in the Golfito district, Southern Costa Rica, around 8 km from the Pacific ocean, in an altitude between 70 and 450 m a.s.l.. The area is located at the corner of Esquinas National Park. The forests were used for logging until recently; this practice has ceased with the establishing of the national park. However, part of it is still primary forest. The rest is secondary forest in different stages of regrowth.

Sampling sites were chosen as "typical" for the respective ecosystems: 2 primary forest sites, one secondary forest site, 2 open grassland sites and a transitional primary / secondary / pioneer location. From each site 5 soil cores were taken and evaluated separately. Corer samples were taken from the corners of a quadrate and the central crossing point to cover the variety of exposition situations at the site, i.e. under different canopy densities and in different distances from trees. The side length of the quadrates was 10-30 m in forest and around 100 m in grassland. The soil cores were taken with a simple cylindrical sampler with 5.2 cm diameter. The top organic layer (in forest only) was collected manually. The dried samples were shipped to Austria and measured by means of gamma-spectrometry. The total stochastic error (1 SD) of the sampling and measuring process is estimated about 20%.

3. Results and Discussion

Both arithmetical mean (572 Bq/m², n = 31) and median (584 Bq/m²) are very close to the value reported by the literature (UNSCEAR), 600 Bq/m². Since a certain fraction of the ^{137}Cs can be anticipated to lie below the sampling depth of 15 cm, the mean total inventory is probably somewhat higher than 600 Bq/m².

The calculated coefficient of variance amounts to 50%, it represents the *total process coefficient of variation*, i.e. including sampling and measuring process related errors. With the latter being about 20% (see above), the *pure spatial variability* of ^{137}Cs (Bq/m²) is about 45% ($0.45=\sqrt{0.5^2-0.2^2}$).

Further statistical analysis (ANOVA, t-test) shows that there is a significant ($p<0.05$) *grouping between forest and grassland sites*, the ^{137}Cs area activity concentration is significantly higher in forest (median: 643 Bq/m², homogeneous within the 4 forest sites) than on grassland (median 291 Bq/m²). The reason for this may be the stronger fixation of Cs in the upper layer of the nutrient poor forest soil as compared to a stronger downward migration on grassland below the sampling horizon (15 cm), a phenomenon which is also encountered in temperate systems, or the more efficient interception of fallout by forest than by grassland vegetation. Also agricultural activity on grassland may result in an increased downward redistribution. However, the mean fraction of ^{137}Cs present in the organic (litter; almost no humus) layer of the forest soils, 0.36% of the total, is much lower than in temperate forests: STREBL et al. (1996) found up to 55% of global fallout ^{137}Cs still being present in the organic layers of Austrian forest soil profiles. The total CV of the forest sites is only 37%, which gives an estimated pure spatial variability of 31%. This figure appears quite similar to a 26% variability found in an Austrian temperate forest (FEA, 1998).

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FATE OF RADIOCESIUM IN FORESTS AND FOREST SOILS: A REVIEW

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1. Introduction

After a radioactive contamination of forest ecosystems, radiation exposure hazard is mainly due to forest lumbering and use of forest products such as foodstuffs. For radiation protection purpose, it is necessary to understand the basic mechanisms of transfer of radionuclides in forests and to identify the main sink-source pools of radionuclides. However, the longevity and heterogeneity of forest stands make difficult the study of the cycling of trace elements in such ecosystems. Some of the available models based on the use of site-specific aggregated transfer factors are capable of reproducing reliable interpretations of ^{137}Cs dynamics in forests (Shaw & Belli, 1998).1999 ? – as in the references ? A complementary analysis of the Cs cycle could be based on a serial approach involving the determination of the key factors and mechanisms acting on Cs mobility at four levels: the forest tree, the soil, the soil clay mineral and the rhizosphere.

2. Forest Tree

Two characteristics of the forest canopy (leaf geometry and biomass) control Cs interception: the key parameter is the foliar index. The rainfall pattern and biological recycling influence the quantity and the nature of Cs activity reaching the soil surface. Further Cs transfer from soil to phytocenosis depends on both the production rate of forest vegetation and soil characteristics. As for the K biological cycle, only a small part of Cs uptaken by trees is immobilized and concentrated in the trunk wood. ^{137}Cs net accumulation can not be dissociated from stem biomass production which is largely age, species and soil dependent.

3. Soil

^{137}Cs rapidly accumulates in the surface horizons of the soil which concentrate 80-90 % of the initial Cs contamination, as measured 2 years after the Chernobyl pulse. The upper soil layers O, OAh and Ah act as a sink but also as a source of ^{137}Cs for contamination of both the phytocenosis and soil biocenosis. The surface horizons of forest soils exhibit large differences in their ability to fix Cs traces. Such variations in Cs mobility are strongly related with the vertical heterogeneity due to the soil 'horizonation'. This heterogeneity is particularly marked in acid soils with thick humus types such as *mor* and *dysmoder*. High Cs mobility is generally associated with acid soils characterized by a relative accumulation of organic matter and an advanced stage of weathering.

4. Soil Clay Mineral

Cs is fixed on the frayed edge sites [FES] (Cremers *et al.*, 1988) of micaceous minerals, the fate of which is affected by weathering. In acid soils, mica transforms into vermiculite and smectite. Al interlayering of these 2:1 expansible clays occurs when Al is not complexed by organic acids. A laboratory weathering model reproducing these processes shows that strong Cs fixation occurs

on vermiculitic sites associated with micaceous wedge zones: this model has been validated in a soil weathering sequence *Cambisol -> Podzol* (Maes *et al.*, in press) and a collection of acid forest soils (SEMINAT, Contract n°FI4P-CT95-0022, European Commission). High estimated values of both the Radiocaesium Interception Potential (R.I.P.) and the quantity of [FES] are associated with soils where the transformation mica -> vermiculite is active. Some of the estimated R.I.P. & [FES] values are higher than those reported in the literature for illitic materials: they are associated with dominant vermiculite. In the soils considered here, the vermiculite content of the topsoil markedly decreases with increasing weathering stage and strongly varies with soil 'horizonation'. Weak Cs fixation in organic rich horizons is due to mineral 'dilution' by organic matter or dominance of smectite. Humus may, however, have a positive effect on Cs fixation by complexing Al and thereby impeding Al interlayering in vermiculite.

5. Rhizosphere

K depletion around plant roots may activate the weathering of micaceous minerals and thereby influence the fate of Cs. The rhizospheric mobilisation of ^{137}Cs using a rhizoplan is correlated, strongly but negatively, with R.I.P.: low rhizospheric mobilisation is associated with dominant vermiculite in soil. Estimating the weathering rate of micaceous minerals in the rhizosphere would therefore provide valuable information to predict the long-term efficiency of the Cs vermiculitic sink in soils.

6. Conclusions

Key forest soil parameters in Cs mobility are: vermiculite and C contents, thickness of organic horizons, acidity and K level. The interaction between soil and forest vegetation has an obvious effect on the Cs cycling which can not be generalized easily. As illustrated here, basic soil processes strongly influence Cs mobility. A realistic development of available models should therefore better take into account a preliminary categorizing of the various forest ecosystems. Such a categorizing would probably better describe the important variability of Cs tranfers depending on soil type, vegetation and climate.

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RADIOCAESIUM DISTRIBUTION IN DIFFERENT COMPONENTS OF AN AUSTRIAN FOREST STAND

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1. Introduction

Observations following the Chernobyl accident revealed that nutrient cycling and storage leads to a much longer persistence of radionuclides in semi-natural environments in comparison with cultivated agricultural land. In 1996 the EC-funded SEMINAT programme was launched to measure and model radionuclide fluxes in forest ecosystems across the European Union. RIFE (Radionuclides in Forest Ecosystems) consists of three models of radionuclide behaviour in forests: one equilibrium distribution model, and two dynamic models with different levels of complexity (SHAW and BELLI 1998).

Within this framework besides sites in Germany, Ireland, Italy and the UK, an Austrian Forest site (Weinsberger Wald) was selected for the acquisition of a site-specific data set of radiocaesium fluxes for the calibration of the RIFE model.

2. Materials and Methods

Weinsberger forest represents one of Austrias largest unbroken managed spruce forests, it is situated in the Granite massiv of Waldviertel (48°23'N, 15°03' E; 900 m alt.; mean annual precipitaion: 910 mm). ^{137}Cs soil inventory: (n = 218, ref.date: 86-05-01): 53 kBq m^{-2} ± 27%.

In addition to data collected within the EC-project ,numerous results of national radioecological monitoring programmes since 1987 are available. Sampling was focussed on the determination of radiocaesium depth distribution in soil profiles, Cs inventory of understorey vegetation (repeated samples of dominant species) and spruce (by felling a single tree and seperate analysis of different parts), ^{137}Cs soil input by litterfall (monthly emptied collection funnels of 1m²) and ^{137}Cs concentration (determined bi-monthly in 25 l samples) in surface water.

3. Results and Discussion

By combination of forest biomass data and the results of gammaspectrometric measurements, the inventory of different forest components was estimated (see Tab. 1).

From these figures it becomes evident that soil acts as an effective sink of radiocaesium. More than 90% are stored to a depth of 15 cm of mineral soil. Derived ecological residence half-times increase from 1.2 and 2.6 to 3.1 years cm^{-1} within the organic matter dominated soil layers (Ol/Of (7.5 - 3.5 cm), Oh (3.5 - 0 cm), OAh (0-5 cm) and decrease to 0.1 y cm^{-1} in 25 cm depth of mineral soil.

Outputs from soil via surface water and tree growth are smaller than annual loss via physical decay (2.27%). Although average ^{137}Cs-contents in dry matter of understorey species are much higher (bilberry: 1482 Bq kg^{-1}, fern: 9341 Bq kg^{-1}(ref. date: 86-05-01) than in tree tissues (bole wood: 66 Bq kg^{-1}, needles '96: 371 Bq kg^{-1} (ref. date: 86-05-01).), due to its low biomass of approximately 50 g m^{-2}, understorey inventory accounts for less than 1% of the total (see Tab. 1). Derived soil-plant transfer factors varied between 0.04 (grasses) and 0.27 m² kg^{-1}, exceeding

typical values for agricultural crops by far (e.g. wheat: 0.0003 $m^2 kg^{-1}$; GERZABEK et al. 1998). The tree compartment contains 3% of ^{137}Cs.

Tab. 1: Estimated radiocaesium inventory of Weinsberger forest and annual changes of soil inventory as determined eleven years after the Chernoyl fallout event

reference date: 86-05-01	Biomass $kg\ m^{-2}$	^{137}Cs - Inventory $kBq\ m^{-2}$	% of ^{137}Cs inventory
organic layer (Ol, Of, Oh)	16	20.7	44
0-15 cm (Ah-horizon)	-	21.2	45
15-30 cm (B-horizon)	-	3.3	7
tree (spruce biomass)	12.5	1.4	3
understorey biomass	0.05	0.2	0.5
total inventory	-	46.8	100
		$Bq\ m^{-2}\ a^{-1}$	inventory change % a^{-1}
Soil input: litterfall		199	0.43
Soil output: surface water		7	0.01
soil output: bolewood increase		11	0.02

4. Conclusions

- Forest soils act as an effective sink for radiocaesium
- ^{137}Cs uptake by understorey plants reveals the high availability of radiocaesium in forest soils
- Organic matter plays a key role in fixation of ^{137}Cs and retards leaching to deeper layers of mineral soil
- Leaching loss rate of ^{137}Cs from soil to surface water is low (0.01 % a^{-1})
- Soil processes and behaviour in organic soil horizons represent a focus of interest in modelling radiocaesium dynamics of spruce forests

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THE INFLUENCE OF CLIMATE ON RADIONUCLIDE BEHAVIOUR IN THE TERRESTRIAL ENVIRONMENT

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1. Introduction

Processes governing the behaviour of radionuclides in the terrestrial environment are affected by meteorological, seasonal and climatic factors in a series of complex interactions with biotic and abiotic components. In models assessing the consequences of a release of radioactive material in the environment, parameters are needed to represent those processes by which the radionuclides enter the human foodchain. Several values of these parameters have been determined for plants and soils of temperate climates, but there is a great area of uncertainty for non-temperate climates. The aim of this paper is to collate information on the knowledge available concerning the effects of climate on foliar and root uptake and to identify priorities for future work.

2. Materials and Methods

Results have been analysed of a survey conducted among leading radioecologists in 1997 on foliar and root uptake in the framework of the UIR/EULEP/EURADOS Action Group on Models and Data, along with the response to a questionnaire circulated during the International Meeting on Influence of Climatic Characteristics upon Behaviour of Radioactive Elements, held in 1997 at Aomori, Japan. Data and models collated in the literature have provided additional information for production of this overview.

3. Results and Discussion

Meteorological events largely affect dry or wet deposition of radionuclides to vegetation (IZRAEL et al., 1996) as well as resuspension (SCHULLER et al., 1995). Information on how meteorological factors affect parameters such as interception, adsorption, absorption and loss has been discussed and reviewed by WATKINS and MAUL (1995), who conclude that some data are very sparse and some processes are not usually accounted for explicitly in mathematical representation of the processes. Data generally refer to temperate climate and do not necessarily fit in with other climatic conditions. The influence of seasonality on the radiological impact of environmental radioactive contamination has been discussed by AARKROG (1992). Seasonality is particularly important when direct contamination is the dominating pathway. In agricultural ecosystems seasonality is generally of greater importance in temperate regions than in subtropics where soils may be cultivated throughout the year.

Precipitation plays a role in the redistribution of radionuclides deposited on the soil through leaching, infiltration and evapotranspiration, but temperature also controls the movement of radionuclides along the soil profile. This has been ascertained at different latitudes and altitudes (GERZABEK et al., 1997; SCHULLER et al., 1997).

Seasonal variations of root uptake of trace elements by plants are ascribable more to the hydrological properties of the soil than to precipitation and air temperature (KIRCHNER and EHLKEN, 1997). Annual variations of soil to plant transfer factors were observed even of a factor three from the mean value (FRISSEL, 1997). Soil to plant transfer differs in areas

characterized by different climate, as a result either of different latitude or of different altitude. The variability of transfer factors among lowland areas, highland areas and alpine pasture at the same latitude is ascribable to different attributes of ecosystems (GERZABEK et al., 1997). The extremely high variability in transfer factors within different climatic areas has been underlined by several authors (FRISSEL, 1997; HARRIS, 1997; LI et al., 1997). From a comparison between soil to plant transfer in subtropical and tropical areas and similar data from temperate areas, FRISSEL (1997) observes that there exist no systematic differences between soil-to-plant transfer factors in temperate, subtropical and tropical environments, but there exist ecosystems with a relatively high or low uptake, whose abundance is much higher in tropical environments than in temperate climates.

4. Conclusions

The available information on the radiological impact of environmental radioactive contamination in different climatic conditions is incomplete. Seasonal and annual variations of radioecological parameters have been discussed, but data generally refer to temperate climate and do not necessarily fit in with other climatic conditions. There is a need for a better understanding of the processes that influence seasonality in natural and seminatural ecosystems and to formulate site specific models. Sources of variability for plant to soil transfer are not necessarily connected with a climate, but with a particular kind of ecosystem. A systematic screening of the abundance of deviating ecosystems therefore has a high priority. Understanding of single isolated processes and parameters in different climate conditions is not sufficient to improve our knowledge. The complexity of the whole ecosystem structure, processes and dynamics has to be taken into account to predict the behaviour of radionuclides under different climatic conditions.

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CS-137 FLUX FROM SOIL TO ROE DEER

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1. Introduction

Roe deer from the state forestry Ochsenhausen in the southwest of Germany, about 200 animals per year, have been measured since 1987 with respect to their Cs-137 activity per kg of muscle meat. The contamination of individual animals varies between a few Bq/kg and several 1000 Bq/kg. The mean soil contamination of the forestry was about 39 kBq/m² of Cs-137 at the 1.5.1986. As the sites are known, where roe deer are shot, we can attribute to each piece of roe deer the Cs-137 inventory of its grazing area. It is known that the approximately 200 roe deer shot per year represent about 1/3 of all roe deer in the forestry, which means that about 600 roe deer live on an area of about 1600 ha of mainly spruce forest.

2. Materials and Methods

Flux is defined here as the movement of activity from soil to roe deer meat as a function of time. Samples of fresh roe deer muscle meat of 10 to 100 g have been analysed gamma-spectrometrically using HPGe detectors. In flux values, the soil inventory is decay corrected to the date of shooting of the roe deer.

3. Results and Discussion

With the knowledge of activities in roe deer and in soil of the grazing area we calculated the flux during a time interval as given in Fig.1. This flux can be interpreted as follows:

- In 1986, the contamination of soil started 1st May, and the transfer to roe deer meat began with grazing of surface contaminated plants like blackberry which have a vegetation period of 12 months. So we assume the period of time for this first flux of Cs-137 into roe deer meat as 12 months and extrapolate from the exponential fit of Fig. 1 a flux of 35 *10-9 per day.
- The flux shown in Fig. 1 is the flux per day from soil to those roe deer, which are shot. The 200 roe deer shot are replaced by young roe deer born in May and June which reach the mean roe deer weight of about 17 kg within one year. The activity lost from soil to the newly born roe deer in their first year is a flux of about the same size as that shown in Fig. 1.
- Since 1987 our data for first halfyears show a slow decrease of flux with an ecological halftime of about 3.8 years. This flux arises mainly from the consumption of contaminated green plants. The decrease of flux with time is caused by loss into other compartments and/or by fixation processes of Cs-137 in the soil e.g. at clay minerals. This decrease amounts to about 18% per year.
- The maxima of flux in fall are mainly due to grazing of contaminated mushrooms, and they depend in size on precipitation in July, August and September. Assuming a period of 45 days of constant flux into and out of roe deer e.g. in 1993, we can estimate from Fig. 1 the value of these two fluxes as roughly 2*10-7 per day.

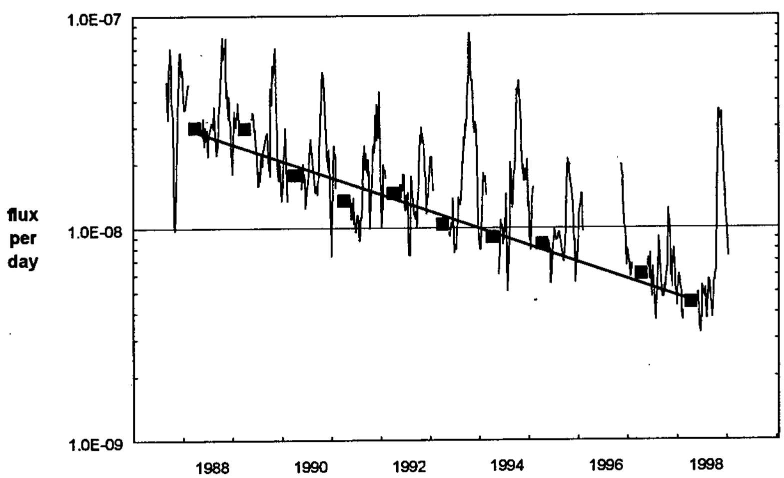

Fig. 1: Cs-137 flux soil – roe deer from Ochsenhausen. Geometric mean values of data 10 days before and after the date are plotted. Exponential fit to geometric mean values of data for first halfyear is also shown.

4. Conclusions

Flux soil–roe deer can be time-dependent because of biological effects e.g. temporal changes of the grazing spectrum, and a decrease of the bioavailability of Cs-137 due to fixation e.g. at clay minerals. Compartment modelling is in progress to determine fluxes in more detail.

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LONG-TERM DYNAMICS OF CHERNOBYL-DERIVED ^{90}SR IN SOIL SOLUTIONS OF FOREST ECOSYSTEMS

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1. Introduction

After radiactive pollution of forest ecosystems radionuclides ultimately reach the soil surface. A portion of the ralionuclides from fallout turns into mobile forms and moves into the soil solution. The concentrations of most long-lived radionuclides in the soil solution are usually quite low because of efficient adsorption of the radionuclides by soil components. Nevertheless, the liquid phase of soil contains the most mobile and bioavailable chemical forms of radionuclides. Consequently, any forecasting of radionuclide transport down the soil profile and into foodwebs is based on analysis of the content of radionuclide soluble forms in soils. The aim of the present investigation was to study the relative content of Chernobyl-derived ^{90}Sr (as one of the long-lived radiopollutants) in soil solutions of forest ecosystems in relation to the physico-chemical properties of radiactive deposition and soil characteristics for years since the accident.

2. Materials and Methods

The soil samples were taken from various subhorizons of forest litter (Ol, Of, Oh), organic-mineral horizon (OAh) and mineral horizons (E, EB) of soddy-podzolic sandy soils at different sites (plot 1,2 and 3) in 30-km zone around Chernobyl nuclear power station (ChNPP) known to differ in radionuclide deposition, soil properties and forest type (Table 1).

Table 1: Soil characteristics and dynamics of RC_{total} values of ^{90}Sr in different plots around ChNPP.

Plot	Locality, ecosystem type	^{90}Sr contamination density, MBq/m^2	RC_{total} values				
			1987	1988	1990	1993	1994
1	6,0 km to the west of reactor, mixed forest	1.9	0.23	1.0	0.36	0.50	0.46
2	6.5 km to the south-east of reactor, pine forest	0.89	0.67	2.3	7.6	8.9	7.2
3	28.5 km to the south of reactor, mixed forest	0.21	3.5	3.1	13.8	14.2	12.5

Samples of air dry soil were sieved to 2 mm, incubated for one week at a water content equal to 60% of the corresponding maximum water capacity. The soil solutions were then isolated by technique adopted for the studies (Agapkina et al., 1995). The samples were centrifuged (4h, 6000 rpm) using a double cup, consisting of an inner cup, which held the soil sample and had a porous bottom covered with a coarse filter papar, and an outer cup for the collection of the soil solution. The relative content of ^{90}Sr in soil solution (RC) is expressed as the ratio of radionuclide activity measured in the soil solution to the total radionuclide activity measured in

the soil sample. The errors (coefficients of varianion) of RC values were generally less than 20%.

3. Results and Discussion

In the initial period immediately after the Chernobyl accident the relative content of ^{90}Sr in the liquid phase of the whole contaminated layer of soil (RC_{total}) was very low and depended chiefly on physico-chemical properties of nucler fallout (Table 1). It is known that considerable part of the Chernobyl radiostrontium was in the form of "hot particles." As the solubility of the "hot particles" is very low and because their quantity and size decrease with increasing distance from reactor, RC_{total} values were much lower at the proximal part of the zone (plot 1 and 2) than that located at a boundary of the zone (plot 3). By 1990 the parameter under study increased (up to 11 times) and then varied in time only slightly over the whole zone. In recent years the variations of RC_{total} values between plots of the zone were related to diversity of soil characteristics. The parameter decreased with increase in content of organic matter, clay content and cation exchange capacity. The studies on long-term dynamics of ^{90}Sr relative content in soil solutions of individual soil layer revealed the variation of RC values down the soil profiles. Since 1988 the minimum value of the parameter was observed in forest litter layer Oh and organic-mineral horizon OAh. This phenomenon was similar to that determined for radiostrontium of the atmospheric fallout (due to the nuclear weapons testing) in forest soils of Ukrain Polessye and may be explained by adsorption of the radionuclide by clay minerals presented in these layers.

4. Conclusions

Since the Chernobyl accident ^{90}Sr deposited on forested areas have involved in soil processes. Three processes controlling the radionuclide transport into liquid phase of soils have taken place all over the zone without regard to soil characteristics and properties of fallout. They are radiostrontium transfer from fallout into soluble forms, its migration down the soil profile and absorption of radionuclide in organic-mineral layer of soil under the forest litter. The combined effect of intensive disintegration of "hot particles" and moderately strong absorption of ^{90}Sr by soil clay minerals (for the most part through the ion-exchange mechanism) resulted in increasing of parameter under study in the first years after accident. The weak variations in RC_{total} values with time for succeeding years allow to suppose that the soil system is coming closer to equilibrium for radionuclide distribution between solid and liquid phases. In the future the relative content of ^{90}Sr in soil solutions of the forest ecosystems and its bioavailability will be mainly influenced by the soil properties and will not depend on properties of primary fallout.

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RADIOCAESIUM OUTPUT FROM THE CATCHMENT OF RIVER TRAUN (AUSTRIA) BY SURFACE WATER

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1. Introduction

The erosion of ^{137}Cs from soil into surface water was studied in a region of Austria which is known for its high ^{137}Cs inventory due to the impact of the Chernobyl accident (FEA 1996). The annual erosion rate has then been compared with the respective result from another investigation area of different size and geographical character.

2. Materials and Methods

The investigated region is part of the Salzkammergut region shared between the countries of Upper Austria, Salzburg and Styria. Its main characteristics are mountains up to almost 3000m, mainly limestone, several lakes imbedded in the river valleys and high rainfall. The largest and deepest lake is lake Traunsee, with an area of 24.4 km², a maximum depth of 189 m and a volume of 2.23 km³. Its main tributary is river Traun whose theoretical residence time in the lake is ca. 1 year. The catchment area until the point where the Traun leaves the lake is 1417 km². However, the drainage area effective for *particulate* erosion is smaller, since further lakes upstream lake Traunsee serve as efficient sediment traps, i.e. represent barriers against sediment transport from further upstream. Therefore, the effective catchment area for particulate transport is only 534 km².

The soil contamination of the region has been measured by means of soil sampling and in-situ-gamma-spectrometry on around 80 measuring points. The total inventory was assessed by interpolation (kriging).

Lake bottom sediments were investigated by taking 24 cores (cores lenghth 50 cm depth) in different parts of the lake bottom. For determination of ^{137}Cs-depth distribution within the sediments the cores were cut in 1-2 cm slices, dried and separately subjected to gamma-spectrometry.

^{137}Cs deposited in the sediments can be interpreted as the total *particulate* input of ^{137}Cs into the lake; the dissolved fraction of ^{137}Cs is not covered by this method, however. This fraction has been assessed by analysing surface water samples of rivers within the catchment area. These water samples (60 litres) were divided into particulate and liquid fraction by a 2 months sedimentation period. After separation, both fractions were analysed by gamma-spectrometry. The results from the lake sediment cores are considered more reliable for the assessment of the particulate transport of ^{137}Cs, since they conserve the"erosion history" of a longer time period in comparison with water samples taken at a single point of time. However, in order to improve the statistical significance of the latter, erosion rates for dissolved ^{137}Cs were calculated not only for the total catchment, but also for several "sub-catchments" related to the respective sampling sites or rivers. The geometric mean was used as final result.

3. Results and Discussion

3.1 Caesium inventory in soil

The inventory of ^{137}Cs in the total catchment area is 75.8 TBq (all activity figures refer to 1 May 1986). The horizontal variability is very high, varying between 10 and almost 200 kBq/m^2. For the area effective for particulate erosion we found 35.0 TBq.

3.2 Caesium in lake bottom sediments

The sediment cores show significant ^{137}Cs maxima in a depth of 2 to 9 cm, depending of the sedimentation rate which varies over the lake according to the path of the inflowing water. A second, but less significant maximum can be found at 10-25 cm depth. Whereas the first maximum can be attributed to the Chernobyl input, the second one is due to the main ^{137}Cs fallout after the atmospheric nuclear bomb testing around 1964. From the depth of the peak and the known time which has passed since the deposition, and neglecting the mobility of Cs in the sediment, a sedimentation velocity of 0.2-0.8 cm/a can be calculated. For the current (=1997) mean annual ^{137}Cs input, calculated from the activity concentration of the surface layer of the cores, we found 197 Bq/m^2.a. Considering the area of the lake, an annual input of 4.9 GBq and, relating this result to the 35 TBq inventory, an erosion rate of 140 ppm per year was derived.

3.3 Radiocaesium dissolved in surface water

The ^{137}Cs concentrations in water of different rivers were between < 0.11 and 1.6 mBq/l, the corresponding erosion rates between < 3 ppm and 48.4 ppm per year.

3.4 Total erosion rates

With the above figures, the mean annual erosion rate for 1997 can be calculated as 8 10^{-6} a^{-1} for the dissolved and 140 10^{-6} a^{-1} for particulate fractions of ^{137}Cs, respectively. The total erosion rate is therefore around 1.5 10^{-4} a^{-1}, most of the eroded ^{137}Cs-activity being transported bound to suspended particles.

For another Austrian region which has been investigated in 1997, a 9.2 km^2 hilly, mostly forested, granite area in Lower Austria, the respective results are 70 ppm per year for dissolved and only 40 ppm per year for the particulate fraction of ^{137}Cs in surface waters, or a total erosion of the ^{137}Cs catchment inventory of 1.1 10^{-4} a^{-1}.

Although the contributions of the dissolved and particulate phases are obviously very different in the two regions, the total erosion rate is quite similar. In comparison with the loss of ^{137}Cs due to radioactive decay (2.27% per year) the determined loss rates via surface water are smaller by a factor of ~ 150.

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BEHAVIOR OF RADIONUCLIDE ^{137}CS IN A WET MONTANE FOREST ECOSYSTEM IN SUBTROPICAL TAIWAN

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1. Introduction

Tremendous amounts of nuclear fission products have been released to the stratosphere through the nuclear weapon testing executing in 1950s and 1960s. In general, radionuclides accumulate more in higher latitudes and those areas with higher precipitation. So far only limited information on the behavior in subtropical and tropical ecosystem is available. The objectives of this study are to identify the accumulation and the fate of ^{137}Cs in an undisturbed wet montane forest ecosystem in Taiwan.

2. Materials and Methods

The distribution of radionuclide ^{137}Cs was intensively investigated in an undisturbed wet montane ecosystem surrounding Yuanyang Lake, which lies at an elevation of 1670 m in northeastern Taiwan. With a mean annual temperature of 13°C and an evenly-distributed annual precipitation of over 4000 mm, this area is occupied by a mossy forest, and thus represents a rare perhumid Temperate locality in Subtropical region.

This study began with a general soil survey, followed by an investigation of the distribution of ^{137}Cs in the forest soil and all other living ecosystem component such as branches, twigs, roots, and leaves, to determine ^{137}Cs cycling in the ecosystem. The radioactivity concentration of ^{137}Cs was determined by □-spectroscopy with a Ge (Li) detector.

3. Results and Discussion

Although the soil is extremely acidic (pH = 3.3 to 3.6) and the rainfall is high, ^{137}Cs is evidently retained in the organic layer. The migration of ^{137}Cs downward from the organic layer to the lower horizons of the soil profiles was negligible. Topography is a critical factor for the distribution of ^{137}Cs. It is shown that the concentration of ^{137}Cs is highest at the foot of the slope and lower near the summit and near the lakeshore. The variation of the concentration along the landscape has been attributed to erosion-deposition in combination with surface run-off of the undisturbed forest. The concentration of ^{137}Cs in the layer of mosses and litter was much lower than that in the organic layer which suggests that the source of ^{137}Cs is not from the newly deposited nuclear fallout. The radioactivity concentration and transfer factor (TF) of ^{137}Cs varied with plant species. The content of ^{137}Cs in the aboveground tissue of understory vegetation reflected more consistently that of the soil than of the conifer. Shrubs and ferns have higher values than a coniferous tree (Taiwan cedar). The TFs in this ecosystem range from 0.21 to 1.88. The high values of TFs are attributed to the abundance of the organic matter in the forest soils. Comparatively, much lower amount of ^{137}Cs accumulated in aquatic ecosystem. Smaller amounts of ^{137}Cs were in sediments than in the forest soil. Only trace amounts of ^{137}Cs were found in the tissue of aquatic plants. Generally the concentrations of ^{137}Cs in this ecosystem are tremendously higher than those in any other places have been studied in Taiwan. The geographic features and the undisturbed ecosystem could give the reason for the accumulation.

4. Conclusions

The rapid recycling of ^{137}Cs through the soil-plant system in this undisturbed multistoried forest ecosystem suggests the existence of an internal cycling that help the accumulation of ^{137}Cs in this ecosystem.

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Table 1. Distribution of ^{137}Cs in the forest soils of the Yuanyang lake ecosystem.

				^{137}Cs radioactivity			
		(g/m^2)		(Bq/kg)		(Bq/m^2)	
Moss		54	± 45	11.2	± 10.3	0.9	± 1.0
Litter		380	± 257	7.4	± 4.6	2.3	± 2.3
Soil	O/A layer[1)]	13710	± 8260	51.7	± 25.2	742.5	± 592.2
	mineral layer[2)]	74800	± 74362	2.3	± 3.3	163.7	± 166.5
Root	Fine	1004	± 682	42.7	± 11.1	47.6	± 47.0
	Medium	569	± 202	24.0	± 15.8	15.1	± 14.5
	Large	1156	± 509	13.6	± 3.9	14.7	± 5.2

[1)] The depth of the soil horizon depends on each individual site

[2)] Only the layer underneath the O/A horizon was included in the calculation

MOBILITY OF CESIUM AND STRONTIUM BY SOIL EROSION BY WATER

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1. Introduction

Following a nuclear accident, farmlands can be contaminated by reactor elements ejected into the atmosphere. The main concern is the transfer to man of the fission products scattered at large distances from the plant. The final goal of this study is to identify and quantify transfer's mechanisms of radioactive contaminants in an agricultural watershed.

Experiments on cesium-134 and strontium-85 speciation dynamics during interrill erosion are based on:

- rain and overland flow simulations,
- speciation trials in batch tests CLAVAL et al. (1997),
- slaking tests LE BISSONNAIS (1998).

In this paper, only the results from the slaking tests are shown.

2. Materials and Methods

The contamination simulator is composed of a ORSTOM. type oscillating nozzle scrubber ASSELINE and VALENTIN (1978), an overland flow injector, and an induction oven. It reproduces the aerosol emitted during an accident.

A 0.5 m^2 tub (1.0 x 0.5 m) is filled with 2 cm layers of neatly rammed soil, up to a 16 cm thickness. The tub bottom is punched with holes and covered with a geotextile to make it more permeable to soil drainage. The overland flow waters were collected at the tub's outlet.
Produced by the scrubber, 1.5 mm drops fell from a height of 2.5 m and reached 90 % of their maximum speed. The rain intensity ranged from 15 to 140 $mm.h^{-1}$.
The overland flow water injector created a sheet of water at the inlet of the soil tub.
The induction oven (POLYR[1] installation) heats a blend of powder up to 2,800°C. This rise in temperature, in a atmosphere in charge of moisture, triggers the formation of aerosols. The aerosols were transferred in a contamination pouch where the soil tubs were placed.

The contamination simulator allows reproduction of 3 types of radioactive particle deposits.
The dry atmospherical deposit was obtained when the soil tub was placed in the induction oven pouch. The wash off was simulated with the scrubber and contaminated water. The last type of contamination was the contaminated overland flow using the overland flow water injector and contaminated water.

Four techniques were used to study the overland flow waters reaching the soil's tub outlet. The collected soil was analysed with a γ spectrometer, to determine the strontium and cesium

activities fixed in the various granulometrical fractions. The solid/liquid phase separation was performed by low pressure frontal filtration with 9 decreasing porosity filters (from 1,000 to 0.45 µm). The fine granulometrical analysis of micro-aggregates and of elementary particles in the overland flow waters was performed with a laser diffraction granulometer (Mastersizer S long bench and automatic liquid channel preparator). The chemical analysis of ions was performed by capillary electrophoresis (Waters WAT 251001 1997).

3. Results and Discussion of the slaking test

During slaking, sorption of cesium and strontium reached equilibrium at 60 minutes with 88 % of cesium and 54 % of strontium sorbed. This time square with the beginning of the effective release of major cations (Na^+: 30, K^+: 8, Mg^{++}: 7, Ca^{++}: 2 % of echangeable fraction). But, after 15 minutes slaking stop effect on the aggregates. Particles generated have mean diameters of 500 or 100 µm.

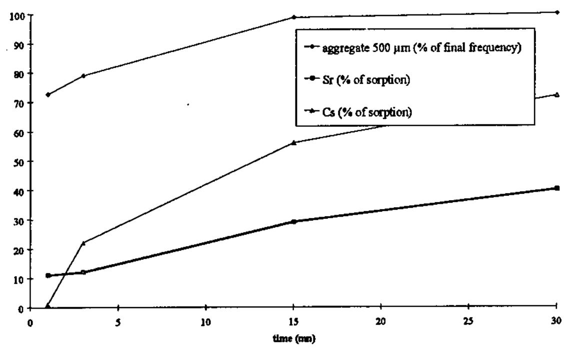

Figure 1: Kinetics of slaking and sorption of cesium and strontium

4. Conclusions

After 15 minutes of slaking, the sorption becomes prevailing on slaking for mobilization contamination to overland flow.

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PLANT UPTAKE OF RADIOCESIUM AND RADIOSTRONTIUM

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1. Introduction

As a result of the accident at the Chernobyl nuclear power plant in 1986 different radionuclides including radiocesium and radiostrontium were deposited over large areas of the European continent. Both nuclides have relatively long half-lifes and are chemically very similar to the nutritional elements potassium and calcium, respectively. On contaminated soils plant uptake can lead to an accumulation of the two radionuclides in the human food chain (Avery 1996). From an environmental point of view radiocesium and radiostrontium are therefore the two most hazardous man-made radionuclides (Shaw and Bell 1994).

Extensive work has been done in determining factors of soil-to-plant transfer of these two radionuclides, but correlations of plant contents versus soil parameters are still quite weak (Smolders et al. 1997). Plant uptake is strongly dependent on the effective availability of the elements for roots in the soil environment (Marschner 1995). Availability for plant uptake from soil solution depends for radiostrontium primarily on unspecific cation exchange, whereas for radiocesium a second, more specific binding to clay minerals is postulated. For both nuclides little is known on the influence of different environmental conditions on root uptake and partitioning within the plant.

Our aims are to find plants that combine high cesium and/or strontium accumulation in the shoot with considerable biomass production. For such species uptake characteristics under different environmental and nutritional conditions should be studied in detail. Plants with well known uptake characteristics for radiocesium and radiostrontium could be used as bioindicators of the effectively available fractions in a contaminated soil. Furthermore such plants might also be used for the remediation of highly contaminated soils.

2. Materials and Methods

To a better understanding of uptake mechanisms root uptake of radiostrontium and radiocesium will be studied in nutrient solution culture but also from contaminated soil. Fast growing and biomass rich plant species should be found that are able to accumulate one or both radionuclides. For a use as a bioindicator the plant should also be able to grow on a wide range of different soil types. Therefore the investigations are mainly focused on different weed species expecially *Amaranthus retroflexus* L. which was reported to accumulate considerable amounts of radiocesium (Lasat et al. 1998).

3. Results and Discussion

For the moment only preliminary results are available. Studies of plant uptake mechanisms are necessary because they are one of the key processes in soil-to-plant transfer either for modeling of radionuclide fluxes and for soil remediation by phytoextraction.

Besides plant uptake characteristics the determination of the effective availability from soil solution is an other important point for this research area. Huge amounts of different extraction procedures are used to determine the availability of compounds from soil solution. Although the use of plants as bioindicators could circumvent the need of extraction methods they are still necessary for "calibration" of the bioindication method.

Especially for radiocesium, the application of the phytoextraction technique on a contaminated soil is difficult because of the strong radiocesium retention on clay minerals (Entry et al. 1996). To get significant results it is therefore necessary to find a way to remobilize the sorbed radiocesium in soil, e.g. by the addition of ammonium (Lasat et al. 1997).

4. Conclusions

To achieve better results either in modeling of radionuclide soil-to-plant transfer or in phytoextration further research in the soil chemical processes and in the plant uptake mechanisms is needed.

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MATHEMATICAL MODEL OF ^{137}CS DYNAMICS IN A CONIFEROUS FOREST

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1. Introduction

Initial data were obtained due to our original studies of the radionuclide dynamics within the so-called 30-km Exclusion Zone of Chernobyl NPP in 1986 - 1994 (Mamikhin et al., 1997). Analysis and formalization of the present information on radionuclide behaviour in terrestrial ecosystems were realised within the framework of the informational-predictive system ECORAD (Mamikhin, 1996).

2. Model Description

The model includes the following main processes running in the system "vegetative cover - soil":
- natural decontamination (plant self-decontamination as a result of the fall of leaves, needle, branches, generative organs, external bark, and washing off by the atmospheric precipitation);
- downward radionuclide transport in the woody plants (from assimilative organs and bark to roots);
- radionuclide release into the soil with the root discharges;
- radionuclide root uptake and upward transport from the soil to the aboveground phytomass.

The algorithm is based on the following assumptions:
- The behaviour of ^{137}Cs principally obeys the same regularities as the behaviour of its stable chemical analogue - K.
- The radionuclide dynamics are considered in its tight connection with the corresponding dynamics of the phytomass.
- Radionuclide content in the vegetation is analysed separately for each of two principal groups: (1) external and (2) internal contamination. The reason for this division is the radionuclide pathway to the plant structures. Internal contamination is due to radionuclide transfer from the soil to plant via root system (root uptake) or radionuclide redistribution within the plant (from more contaminated parts of the plant to the less contaminated ones). The external contamination is fully determined by direct radionuclide fallout onto the aboveground organs of plants exposed.

Validation of the model was made using the original data.

3. Results and Discussion

The model allows simulation of long-term dynamics of ^{137}Cs content in the components of coniferous forest ecosystems of landscapes with different degrees of moisture status and in different conditions of fallout of small-size radioactive precipitation. The situation of fallout in the form of dry aerosol was imitated (deposition of ^{137} Cs: 50 kBq /m^2 at the top of the canopy).

Table 1: A forecast of ^{137}Cs content in the Pine trees of eluvial landscape for 20 years ahead based on our model (Bq of ^{137}Cs / kg dry mass).

	Years after fallout			
Parts of Pine trees:	2	5	10	20
Bole wood	79.9	51.2	29.8	10.6
Needles	2068	919	542	183
Bark	2198	925	312	84.3
Branches	633	171	99.7	35.7
Large roots	370	255	147	52.5
Small roots	840	771	482	174

Some problems attributed to the modelling of the radionuclide dynamics in forest ecosystems were revealed in the course of model development. Of particular importance is in our opinion to take into account the biological availability of the radionuclides when describing ^{137}Cs uptake by plants. This task was accomplished only to a first approach, by introducing the time function of this parameter (availability dynamics). This somewhat limits the range of the model application. More general description of ^{137}Cs root uptake can be obtained taking into account the effect of physico-chemical soil properties on the processes of radionuclide sorption by soil. The present model-calculated dynamics of the root contamination cannot be considered as fully reliable because of the lack of experimental (field) data to calibrate the model and verify the adequacy of the simulation.

4. Conclusions

The model algorithm makes it possible to simulate reliably the conditions of forest ecosystem contamination by radioactive caesium as a result of emission of radioactive matter to the atmosphere (pulse or for a range of years). Now the model is under further development and upgrading. The function of biological accessibility is being modified. The range of application of the model is broadening by including the fuel component into the list of state variables. This will make it possible to simulate radioactive fallout in the form of large particles as well as small (aerosol) ones. A family of the similar models will be developed to describe radionuclide behaviour in deciduous and mixed forests.

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MODIFICATION OF RADIOCESIUM TRANSFER FROM SOIL TO PLANTS

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1. Introduction

One of the main aims of the radionuclide uptake investigation is working out of the operating methods decreasing on the one hand radionuclide transfer from contaminated soil to the edible parts of agricultural and increasing on the other hand the radionuclide removal with the green mass yield of agricultural crops or wild species. At the present time there are some methods permitting to modify the soil-to-plant radionuclide transfer to a certain extent. However up to now it is impossible to obtain the essential radionuclide accumulation by plants permitting their using for the purpose of phytodecontamination. All the existing methods of radionuclide uptake modification are directed only to the soil cultivation (liming, deep ploughing and so on). In our opinion the radionuclide uptake optimization (maximization or minimization) is impossible without investigation of radionuclide uptake dynamics and the dynamics of the modifying factors influence. Moreover it is necessary to investigate the dose dependence of each factor influence on the process of radionuclide uptake and to take into account the possible nonadditive effects (synergism, antagonism) when we use several factors simultaneously.

2. Materials and Methods

The influence of 5 factors on the radioceasium uptake and its removal by Zea mays and Vicia faba with the yield has been investigated in the field experiment in 10-km zone of Chernobyl Nuclear Power Station (ChNPS) (Mikheev et. al., 1992). The reason of factor selection was following. The acute gamma-irradiation of seeds before sowing is capable to change the plant growth processes for a long time after irradiation. As a result the absorption properties of the plant tissues and consequently the radionuclide uptake can be modified (Grodsinsky et al., 1990; Kostyuk et. al., 1993; Mikheev et al., 1995). The employment of the complex mineral fertilizer containing N, P and K must increase the output of the biomass and subsequently the removal of radionuclides from the soil. The purpose of the adsorbent application was to fix the soluble components of the radionuclides in the soil. The peat was used owing to the organic acids which were contented in it and which could fix (adsorb) the radionuclides especially Sr-90. The use of the additional irrigation was based on the results of the laboratory experiments with plants growing in water cultures.

3. Results and Discussion

The influence of all the factors on radioceasium uptake and its removal with the yield of Zea maize green mass was mainly additive except the initial period of the plant growth when the positive interaction between the mineral fertilizer and such factors as acute gamma-irradiation, adsorbent and peat was obtained. The interaction between the mineral fertilizer and irrigation was also nonadditive at this stage of plant growth but its contribution to the radioactivity of the maize plants was negative.

These factor combinations modified the radioceasium accumulation in Vicia faba too much more extent as compare with Zea maize. The majority of the factors (except irrigation) interacted as the antagonists because their independent influence had the same direction and was positive on the one hand and the total modifying effect of the interacting factors was less than the sum of their independent effects on the other hand. The acute gamma-irradiation of seeds and the peat interacted as synergists. At the end of the bean vegetation period these factors intensified the stimulating influence of each other.

The total removal of radioceasium has been increased also under the influence of the irrigation but only at the beginning of the vegetation period. It should be noted that Zea maize plants reacted essentially to the used factors at the initial period of the vegetation.

The addition of the peat and adsorbent to the soil resulted in the effects being like those for Zea maize. The acute gamma-irradiation of seeds affected the transfer factor and the total removal of radioceasium which was opposite as compare with those for Zea maize. The addition of the mineral fertilizer led to decreasing of the radioceasium transfer factor and its total removal. The influence of the irrigation was not constant.

4. Conclusions

Thus it is necessary to take into account the obtained data and to choose the appointed part of the plant vegetation period and the direction of each factor influence in order to successfully use the factors increasing the radionuclide uptake with the purpose of the phytodeactivation of contaminated soils.

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UPTAKE OF RADIOCAESIUM BY ROOT AND DISTRIBUTION BETWEEN ROOTS AND SHOOTS

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1. Introduction

The presence of radiocaesium in soils, a radionuclide with a relatively long half-live is observed and its behaviour has been studied for many years. This radionucleide has been introduced in the environment essentially by atmospheric nuclear tests (1950-1960) and the Chernobyl accident (1986). The transfer of this element from soil to plants is one of the principal means of entry into the food chain. In soils, the bioavailability of caesium is essentially determined by its adsorption (Coughtrey & Thorne, 1983, Kirk & Staunton, 1989). Observations *in situ* and in the laboratory have shown differences in soil to plant transfer with various plant species on the same soil. Our aim is to investigate whether these differences are due to root uptake or to redistribution of caesium within plants.

2. Materials and Methods

Plants are growth in a special cropping device which allow soil-plant exchange but without direct contact. The soil used is a sandy-silt with a pH of 6.7 and a CEC of 146 mol_c/kg. Its distribution coefficient, Kd, in adsorption is $3.4x10^3$ dm^3 kg^{-1}; this value is intermediate in the range of observed soil Kd value (*e.g.* 10^2 -10^5 dm^3 kg^{-1} in Smolders et al, 1997). Young plants were placed in contact with soil containing 40 kBq g^{-1} ^{137}Cs for 7 days. After contact, the activity in plant roots and shoots was measured by liquid scintillation on acid extracts. Plant species used were typical of edible plant parts which contribute, directly or indirectly, to human food (rape, red fescue, sheep fescue, wheat, tomato).

3.Results and Discussion

Total quantity of radiocaesium in plants varies little between all the species, as seen in figure 1. There is a correlation between the activity taken up and plant growth during the period of soil-plant contact (data not shown). However, the variation in the distribution between roots and shoots is greater. The proportion of absorbed ^{137}Cs which has been transferred to the shoots varies from 0.23 for red fescue to 0.58 for rape. Differences in translocation may thus be more important than root uptake. Part of the reason may lie in differing growth patterns.

The same data may be expressed as the ratio of concentrations of ^{137}Cs (Bq g^{-1} dry matter) in shoots and roots. Figure 2 shows the relation between ^{137}Cs distribution in plants and ressource allotment for growth of shoots and roots. If the redistribution of ^{137}Cs was determined by biomass production, then the ratios of the amount of ^{137}Cs in shoots and roots would be linearly related to the corresponding ratio of dry matter mass, whereas the concentration ratio would be constant. Figure 2 shows that this is not the case. In general, the ratio of ^{137}Cs concentrations in shoots and roots (C_S/C_R) tends to increase with decreasing mass ratio (M_S/M_R). Concentrations in root are always greater than in corresponding shoots. Ratio between root and shoot

concentrations differ with species and vary by a factor of 7. Variations are also seen in a same genus (a factor of 2 difference between the 2 fescue).

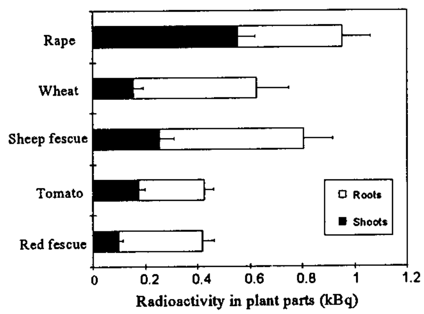

Figure 1. ^{137}Cs found in roots and shoots of various plants after 1 week of growth in contact with contaminated soil (40 MBq kg^{-1})

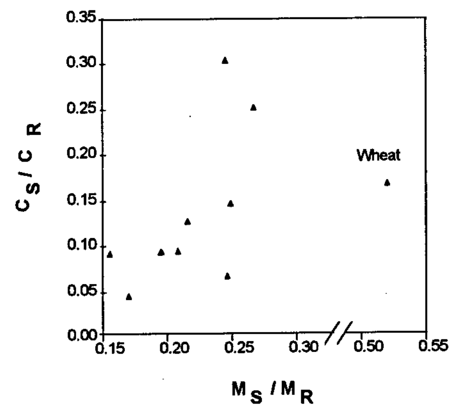

Figure 2. Relation between the ratio caesium concentration in shoots and roots and the corresponding mass ratio.

4. Conclusions

We have seen differences in the radiocaesium distribution between roots and shoots in various plants, with no direct relation with plant biomass. Most of the absorbed ^{137}Cs remains in the roots. Much of the observed differences in soil to plant transfer probably arises from variation in the translocation of ^{137}Cs after root uptake. This is therefore an important factor to investigate in studies of contamination of the food chain.

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Acknowledgements

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ROOT UPTAKE OF ^{137}CS, STABLE CS AND K FROM PADDY SOILS IN RICE PLANTS

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1. Introduction

Radionuclides released into the environment reach the human body through several transfer processes. One important process is the transfer of radionuclides through the soil-plant-human pathway (ICRP, 1987). Nuclear fuel cycling facilities are partially operational and are still under construction in Rokkasho-mura, Aomori Prefecture, therefore, site-specific parameters are important for more precise and realistic estimation of internal radiation dose. Although rice is one of Japanese staple foods, there is insufficient data for the site-specific transfer parameters. In this study, concentrations of ^{137}Cs, stable Cs and K were measured for soil and rice plant samples collected from paddy fields. Root uptake of ^{137}Cs and stable Cs from the soil to the rice plants and the distribution of ^{137}Cs, stable Cs and K in the rice plant components were investigated.

2. Materials and Methods

Rice plant and soil samples were collected from paddy fields in Aomori Prefecture, Japan in 1996 and 1997. After the rice plant samples were separated into several components (polished rice, rice bran, hull, etc.) for analysis of stable elements, they were dried at 50°C and cut into small pieces, and/or were ashed lower than 450°C. The rice plant samples for the analysis of ^{137}Cs were ashed lower than 450°C. The soil samples were dried at 50°C and then passed through a 2 mm sieve. The concentrations of stable elements in both rice plant and soil samples were determined by inductively coupled plasma-mass spectrometry and atomic absorption spectrometry, and those of ^{137}Cs were measured with a Ge gamma-ray detector connected to a multichannel analyzer.

3. Results and Discussion

The deposition of ^{137}Cs in Japan was mainly derived from the global fallout from nuclear explosion testings until the 1980s. The range of ^{137}Cs concentration in paddy soils was 2.5-21 Bq kg^{-1} dry wt. and the vertical distribution of ^{137}Cs was found well within the rooting zone of the rice plants. Similarly, stable Cs and K were also uniformly distributed. Concentrations of ^{137}Cs in polished rice were in the ranges of 2.5-85 mBq kg^{-1} dry wt. The geometric mean of transfer factors from paddy soil to polished rice was 0.0016, which was several times higher than that of stable Cs as shown in Figure 1, and the 95% confidence interval was in the range of 0.00021-0.012. Similar observations were reported by Komamura and Tsumura (1994), Tsukada et al. (1998), and Tsukada and Nakamura (1999). Transfer factors of both ^{137}Cs and stable Cs showed negative correlation with the concentrations of K and stable Cs in the soils. The concentration ratios of ^{137}Cs to stable Cs indicated a relatively constant value in rice plant components, however, those of stable Cs to K were different in the leaf position (Figure 2).

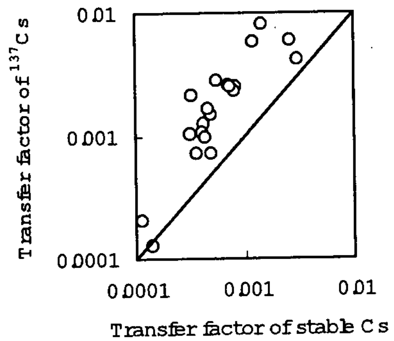

Fig. 1: Comparison of soil-to-polished rice transfer factor between stable Cs and ^{137}Cs.

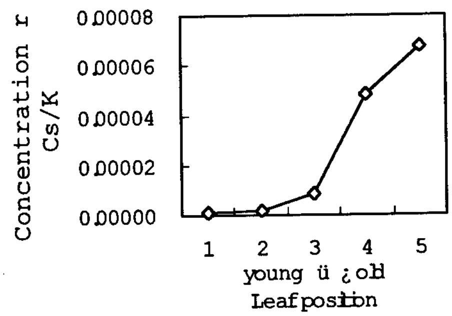

Fig. 2: Concentration ratio of stable Cs to K in rice leaf position.

4. Conclusions

It was found that the transfer factor of ^{137}Cs for polished rice was higher than that of stable Cs. This may be attributed to the higher biological availability of ^{137}Cs in the soils compared to stable Cs. The transfer factors significantly correlated and were dependent on the concentration of K in the soil. These results suggest that the investigation of the root uptake of stable Cs and K in the rice plants could lead to a better understanding of the transfer processes of radiocesium in paddy fields.

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SPECIATION OF RADIUM IN URANIUM MILL TAILING : STUDY OF CHEMICAL EXTRACTIONS

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1. Introduction

Since the mobility of toxic elements, as Pb or Cd, depends on the solid phases where they are fixed (1-3), the speciation of toxic elements has been extensively investigated (1-8). For this purpose, sequential extraction procedures have been proposed and widely used to determine the distribution of trace elements in soils or sediments. These protocols fractionate of total trace toxic elements according to the type of solid phases (clay, organic matter, and metallic oxides) where they are fixed; each different solid compound is attacked successively, with different specific chemical reagents (4,3). The aim of this work was to assess the applicability of such approach to determine radium speciation in uranium mill tailing whose solid compounds are similar to those in soils. Two different acetate salts, commonly proposed in the literature (9), were used to assess the radium bound to carbonated phases, which are made during alkaline treatment of uranium ore and which are the most common acid soluble phase in uranium mill tailing.

2. Materials and Methods

Following chemical extraction procedures have been carried out and compared: 1g of solid phase and 50ml of different reagents: AcONa or $AcONH_4$ (0.5M or 1M), and the mix $AcONH_4$ (0.5M) + NaCl (0.5M) were shaken for 24 hours. After this equilibration time, determined by kinetic study, the solution was filtered and acidified for analysis. The radium activity in solution, using gamma spectrometry, and the total carbonates in solid phase, using Bernard calcimeter method, have been determined for each different experimental condition.

The effect of the pH of extracting solution and the reagent concentration were investigated in order to select the most reliable extraction condition to determine the role of carbonates in radium retention.

Table 1 gives the main characteristics of uranium mill tailing; table 2 compares the amount of Ra extracted and the amount of carbonates removed by each tested medium.

3. Results and Discussion

According to the comparison of the amount of radium released into the solution and the amount of carbonates removed from the solid phase, this work has permits to see some limits in the application of sequential extractions: readsorption during batch experiments or simultaneous attack of different compounds of solid phases have been experimentally shown. In addition, Na/Ra exchange could be exhibit, proscribing the use of sodium salt for Ra speciation in our study. Consequently, the most selective conditions, among those tested, seem then to be an ammonium acetate medium. According to these selected operational conditions, about 1/3 of radium would be fixed on the carbonated phases in the uranium mill tailing studied and would be readily available after carbonates destruction, by acidification for instance.

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Table 1: Main physical chemical features of uranium mill tailing.

pH	Ca ($mg.g^{-1}$)	Mg ($mg.g^{-1}$)	Pb ($\mu g.g^{-1}$)	As ($\mu g.g^{-1}$)	Ra ($Bq.g^{-1}$)	U ($Bq.g^{-1}$)	Carbonates (%)	Quartz (%)	OM (%)
8.58	38.3	32.2	79.6	518	3.7	1.2	11.65	31.0	0.56

Table 2: Evolution of amounts of Ra released from sample (Bq/g) and carbonates removed (percent of total content) for different extracting conditions - with pHe as pH at equilibrium.

		AcONa		$AcONH_4$	
		0.5M	1M	0.5M	1M
pHe=6.1	A_{Ra} (Bq/g)	2.8	2.9	1	1.92
	Carbonates removed (%)	47	43	27	29
pHe=5.00	A_{Ra} (Bq/g)	3.2	3.25	1.1	2.6
	Carbonates removed (%)	69	77	42	69
pHe=4.05	A_{Ra} (Bq/g)	3.52	3.26	2	2.95
	Carbonates removed (%)	100	100	98	98
pHe=3.3	A_{Ra} (Bq/g)	3.67	3.5	2.75	3.28
	Carbonates removed (%)	100	100	98	100

INFLUENCE OF PH AND MONOVALENT IONS ON CESIUM UPTAKE OF H. CRUSTULINIFORME AND P. FORTINII IN BATCH CULTURES.

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1. Introduction

Radiocesium is one of the most significant isotopes deposited in terrestrial ecosystems by nuclear fallout because of its very long half-life of 30.2 years.

In semi-natural ecosystems radiocesium is not leached but still present in the superficial horizons of soil. Results from forest research projects point to a recycling process of radiocesium in which fungi are suggested to play a key role. Fungal mycelia have the capacity to absorb and translocate radiocesium, which is confirmed by the high activities found in fruiting bodies of basidiomycetes (BRÜCKMANN & WOLTERS, 1994). It is supposed that fungi actively accumulate radiocesium and that they may retain up to 40% of cesium in soil (BAKKEN and OLSEN, 1990).

Knowledge of regulation of radionuclide uptake by fungi at molecular, cellular and organismic level could help to estimate their contribution to the radiation exposure of people.

2. Materials and Methods

Phialocephala fortinii as saprophytic and *Hebeloma crustuliniforme* as symbiotic mycorrhizal fungi were chosen for this study.

Uptake was measured in 50 ml liquid batch cultures of MES-buffered MMN medium (Modified Melin-Norkrans) and the pH was ajusted by tetramethylammonium hydroxide. For experiments regarding the influence of monovalent ions the concentration of potassium and ammonium was altered.

After incubation at RT for one week, cesium chloride tracered with ^{134}Cs was added to a total concentration of 50 nM. During a period of two weeks mycelia were regularly harvested. The pH of the medium was recorded and dry weights of the fungi were determined by drying to constant weight at 60° C. Cesium in the solution was measured by Liquid-Scintillation-Counting.

3. Results and Discussion

As it is shown in Fig 1 the uptake by *P. fortinii* exceeded that of *H. crustuliniforme* by more than one order of magnitude. Both species showed pH dependence of cesium uptake. The net influx of cesium decreased when the pH was lowered. At pH 4 the uptake by *H. crustuliniforme* was below the detection limit. During the period studied, *P. fortinii* took up 44 % of the total cesium at pH 6 and 30 % at pH 4. *H. crustuliniforme* absorbed 0.6 % at pH 5 and showed no uptake at pH 4.

First experiments show that in the absence of potassium the initial uptake rate by *P. fortinii* and *H. crustuliniforme* increased and the net uptake results in up to 80 % of the present cesium, while lack of ammonium did not seem to have any influence.

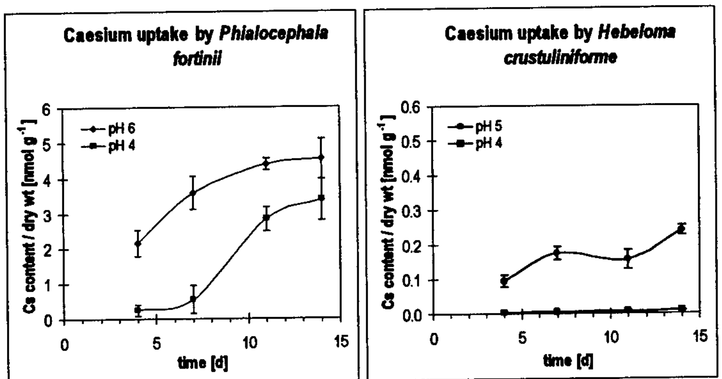

Fig. 1: Plot of cesium content versus time of *P.fortinii* and *H. crustuliniforme* in liquid batch cultures of MNN medium supplemented with 50 nM cesium chloride at RT.

4. Conclusions

Accumulation of substances in cells requires active, energy dependent transport. In yeast cells uptake of the cesium analog potassium is enhanced by glucose and energized by the proton motive force (BORST & PAUWELS, 1981). It is suggested that cesium uptake is mediated by potassium transport systems, these systems being unable to discriminate between the two alkali cations.

Low pH values in the medium disturb the H^+ distribution between the inside and the outside of the cell. This might lead to net H^+ influx causing depolarization of the cell membrane and decrease of cesium uptake. Additionally, protons could inhibit cesium transport by shielding of negative head groups of the cell membrane or direct interactions with the modifier site of the transport protein.

First experiments to examine the influence of monovalent cations potassium and ammonium on the cesium uptake in *P. fortinii* and *H. crustuliniforme* show an increase of cesium accumulation in the absence of potassium. This suggests that the transport system mediating cesium uptake could be identical with the system for other monovalent cations.

The apparently higher accumulation potential of the dark septate endophyte *P. fortinii* compared with *H. crustuliniforme* may be attributed to melanin content of the mycelium. Melanins possess many oxygen containing groups which can serve as potential binding sites for metal ions, thus preventing cesium from flowing out of the cell again.

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Trace Elements and Pedology

(Special Symposium 3)

SMALL-SCALE CHEMICAL HETEROGENEITY IN SOILS: DISTRIBUTION OF METALS IN AGGREGATES

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1. Introduction

As a consequence of anthropogenic inputs heavy metals accumulate in forest and agroecosystems (Meyer, 1981; Wilcke and Döhler, 1995). To evaluate whether these inputs endanger ecosystem functions, information on metal availability for organisms and leaching are necessary (Brümmer et al., 1986). The availability depends on the form and location of metal bonding in soils (Wilcke and Kaupenjohann, 1997). The objectives of this work were to examine whether there is a small-scale heterogeneity of metal concentrations in soil aggregates and to develop hypotheses to explain possible chemical disequilibria.

2. Materials and Methods

Aggregates (2-20 mm) were selected manually from the field-moist samples of 26 horizons in Central Europe and 18 horizons in Costa Rica. The aggregates were separated into "exterior" and "interior" fractions using the method of Kayser et al. (1994). The aggregates were deep-frozen using liquid N_2 and placed on a 2-mm sieve inside a stainless-steel container which contained 300 ml of deionized water. This quantity resulted in a water level of about 5 mm below the sieve. The container then was horizontally shaken. During this procedure the aggregate exterior separated itself from the frozen interior and was collected in the container.

3. Results and Discussion

Total concentrations of mainly geogenic metals (Al, Fe, Cr) are generally smaller in the aggregate exterior than in the interior. In contrast, the concentrations of anthropogenically introduced metals are often larger in the aggregate exterior than interior (Figure 1). In most of the studied temperate soils, metals bound in silicates are depleted in the aggregate exterior, whereas more easily extractable metal fractions are enriched (Figure 2). To explain this metal distribution Wilcke and Kaupenjohann (1997) proposed two hypotheses: (1) Preferential weathering of aggregate surfaces causes a shift from residual to other metal forms and (2) preferential sorption of deposited metals by the aggregate surfaces leads to a total enrichment of metals in the aggregate exterior, especially in easily extractable forms. Hypothesis 1 is supported by the finding that in the tropical soils from Costa Rica total and particularly strongly bound metal concentrations are, on the average, larger in the aggregate exterior than in the interior. In contrast to temperate soils, the preferential weathering of the aggregate exterior in humid tropical soils results in a relative depletion of Si from silicates ("desilication") and a following relative enrichment of Fe and Al oxides which strongly sorb heavy metals (Figure 3, Wilcke et al., 1999). Hypothesis 2 is supported by findings along deposition gradients. Wilcke and Kaupenjohann (1998) showed, that the differences between aggregate exterior and interior are the more pronounced the higher the deposition input is (Figure 4).

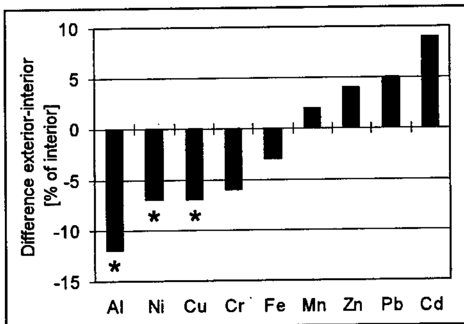

Figure 1: Differences in metal concentrations between aggregate exterior and interior in % of the interior concentrations of 26 temperate horizons (* differences are significant at P < 0.05).

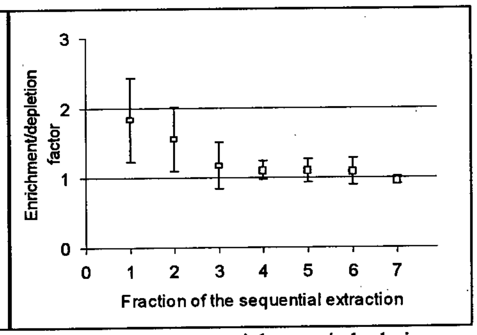

Figure 2: Average enrichment / depletion factors (= exterior/interior ratio of the portion (in %) of a metal concentration in a specific fraction) of all studied metals in 26 temperate horizons and standard deviations.

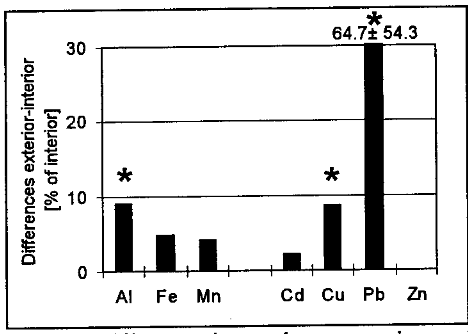

Figure 3: Differences in metal concentrations between aggregate exterior and interior of 18 Costa Rican horizons (*differences are significant at P < 0.05).

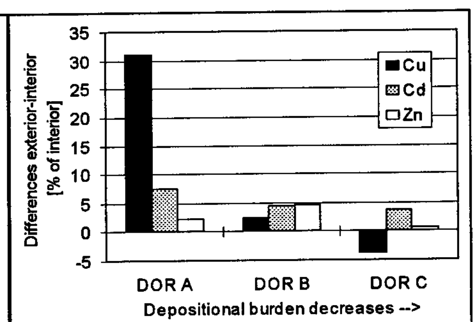

Figure 4: Differences in metal concentrations between aggregate exterior and interior of 3 A horizons along a deposition gradient caused by a brass foundry near Dornach/CH.

4. Conclusions

Metal concentrations are unevenly distributed across aggregates. The distribution may be explained by the combined effect of preferential weathering of aggregate exteriors and preferential sorption of introduced metals at aggregate surfaces.

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STOCKS AND FLUXES OF TRACE METAL ELEMENTS IN A NATURAL FOREST ECOSYSTEM ON VOLCANIC ASH.

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1. Introduction

Knowledge of metal stocks and fluxes in forest soils is of great concern in environmental research, and more particularly for risk assessment. In order to estimate long term effects of trace metal elements originating from anthropic activities, it is necessary (i) to distinguish these sources from the natural soil pools; (ii) to estimate cycling rates of trace elements as a function of soil forming processes. Andosols are known for their abundance in Cu and Zn and also their high contents of Ni and Cr (Shoji *et al.*, 1993). Andosol characteristics include dominance of neoformed amorphous aluminosilicates (allophanes) and high incorporation rates of organic matter. Both constituents are known to be strong metal adsorbents (Clarck and McBride, 1984).

The aims of this study were: (1) quantification of metal stocks in different soil horizons of a young andosol; (2) quantification of deposition and biomass cycling of elements (C, Si, Al, Fe, Zn, Pb, Cu); (3) study of differences in concentration of elements in soil solution percolating the different soil horizons; and (4) assessment of pedological redistribution of metals.

2. Materials and Methods

The forest ecosystem we studied is located on the east slope of the Puy de la Vache volcano (Chaîne des Puys, Massif Central, France). The soil is an andosol developed on volcanic ash under Norway pine trees (*Pinus sylvestris*). Granulo-densimetric fractionations were performed on each horizon to separate soil constituents (Ducaroir and Lamy, 1995). Chemical and physical analyses were performed on both bulk samples and particle size fractions for each horizon. Stocks were calculated using element concentrations, bulk density and thickness of horizons. Polyethylene (PE) zero-tension lysimeters (gutters) were inserted by force into the soil, under Ah2 and B-horizons. Gravitational solutions and throughfall were collected after each major rainy period. pH, total contents of soluble carbon, and trace metal elements were measured in each sample after filtration at 5 µm. Water balances of gravitational fluxes were calculated using the model of Granier *et al.* (1995) using data on total rainfall, evapotranspiration, bulk density, available water capacity and element concentrations of percolating soil solution.

3. Results and Discussion

<u>Stocks</u> (table 1):

During weathering, fate of trace elements depends to a large extend on their initial location in the rock mineral constituents and on pedological processes. Stocks of Si, Al, Fe, Cu, and Zn were lower in the soil than in the parent rock material (S/R < 1). The loss of Si corroborates andosol pedogenesis. Cu and Zn, which were initially dominantly located in volcanic glass, were redistributed, after weathering, on secondary constituents (the finest grain size fraction). Stocks of Ni and Cr were similar in the soil and in the parent rock material. Ni and Cr were dominantly present in olivine and titanomagnetite minerals. Weathering of these minerals mainly consisted in physical breakdown. Therefore, Ni and Cr remained in primary mineral phases. Stocks of Pb were

higher in the soil than in the parent rock material. Pb accumulation was important in Ah1 and Ah2, ascribed to atmospheric deposition.

Table 1: Comparison of stocks in soil and in a comparable thickness of parent rock material (S=A+B+B/C horizons)

Horizon	*Si*	*Al*	*Fe*	*Cu*	*Zn*	*Ni*	*Cr*	*Pb*	**THICKNESS**	*B. density*
	kg/m^2	kg/m^2	kg/m^2	g/m^2	g/m^2	g/m^2	g/m^2	g/m^2	m	kg/m^3
Ah1	11	6.2	6.4	1.8	9	5.4	12	0.62	0.14	520
Ah2	23	14	14	3.4	19	11	27	1.20	0.26	590
B	16	8.9	8.1	2.9	9.4	7.9	17	0.30	0.15	600
B/C	5.7	2.9	3	1.3	3.2	2.7	5.8	0.07	0.05	630
R	92	37	36	21	49	30	66	0.57	0.60	700
S / R coefficient	0.61	0.86	0.89	0.45	0.82	0.91	0.96	9.89		

Fluxes (table 2):

Percolation of Si was important under A and B-horizons. Important amount of Carbon leached under the B-horizon. Calculated throughfall fluxes of Cu and Zn were important. However, Pb and Cu were not transferred in soil solution and only very few Zn is detected. These elements were trapped by secondary constituents such as allophanes and organic matter. Cu and Zn are nutrients and are biocycled. A part of throughfall fluxes of Cu and Zn may be due to the internal cycle in this forest ecosystem.

Table 2: Calculated fluxes of elements in throughfall, in gravitational soil solutions under both A and B-horizons.

Fluxes	*throughfall*	*under A*	*under B*
		$mg/m^2/year$	
Si	6.6	1345	202
Al	1.9	200	7.4
Fe	0.6	67	0.0
TOC	9935	16237	4577
TC	10604	16738	6981
Cu	102	0.0	0.0
Pb	6.8	0.1	0.0
Zn	121	3.9	1.6

4. Conclusions

Following mineral weathering, Zn, Cu, and Pb are dominantly associated to secondary soil constituents. Ni and Cr remain associated to primary mineral phases. Zn, Cu, and Pb leachability is very low. After an initial stage of leaching weathering conditions, today's soil behaviour leads to a relative accumulation of Al, Fe, Zn, Cu, Ni, Cr, and Pb. There is a loss of Si and C. This combined laboratory and *in situ* field study on stocks and fluxes of trace metals revealed different distribution after weathering and highlighted the strong metal retention in the Andosol ecosystem.

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PEDOLOGY AND HEAVY METALS - A REGIONAL APPLICATION IN ITALY

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1. Introduction

Research on distribution and circulation of heavy metals in different environmental contexts, since the last decades, has received a remarkable attention from various scientific domains (e.g. geology, pedology, chemistry, agronomy, botany, pharmacy), with the following finalisations:

- background knowledge and soil genesis;
- environmental contamination;
- soil remediation;
- toxicology and human health.

Heavy metals are "dispersed" trace elements with generally limited concentrations in the earth's crust and the related soils. However, metal accumulation in the environment may occur at some locations, owing to different sources (*Angelone & Bini,* 1992). Possible "natural" accumulation may be related to heavy metal-bearing rocks (e.g. Ni and Cr in serpentine) or to mineralised areas (e.g. Pb and Zn from mixed sulphurs mines), while anthropogenic accumulation is related to industrial activities (e.g. Cd in metallurgy, Cr in varnish and leather factories), agricultural and urban sewage sludge (e.g. Zn and Cu from fertilisers, Cd, Pb, Cr from sludge).
Especially the last item is paying great attention at present, since increasing quantities of USR are produced and extensively introduced in the environment. Therefore, identification of the sources responsible for soil contamination is an important issue, especially for the Environmental Protection Agencies.
Following this approach, in recent years the Environmental Protection Agency for the Veneto Region carried out several soil surveys in agricultural and forest areas of the Venetian territory, with the aim to evaluate the background level of heavy metals in soils, possible contamination of some sites, and the related environmental hazard, with special reference to the Venice lagoon pollution.

2. Materials and Methods

Approximately 400 soil samples from more than 100 representative soil profiles (mostly Inceptisols) were analyzed for total (aqua regia) and DTPA-extractable Cu, Ni, Pb, Cd, Zn, Cr, As, Co, Hg. Analyses were carried out by AA spectrometry on the fine fraction (<2mm) of the soils previously selected. In the same samples, pH, CEC, Clay and Organic Matter percentage were determined with standard methods. The recorded data were statistically processed in order to evidence simple/multiple correlations between variables pairs, and among principal components.

3. Results and Discussion

The mean values of pH in the soils investigated is 8.1, Clay 40%, Organic Matter 3.4% and CEC 41 cmol(+)/Kg. The soils present high levels of anthropogenic heavy metals, with mean values of total As 10 mg/Kg (range 6 – 30), 41 mg/Kg total Cu (range 21 – 74), 80 mg/Kg total Zn (range 63 – 97), 79 mg/Kg total Ni (range 49 – 87), 56 mg/Kg total Cr (range 44 – 65), especially concentrated in surface horizons. Pb (mean 12 mg/Kg), Co (mean 10mg/Kg) and Cd (mean 0.2 mg/Kg) present higher concentrations in organic horizons than in mineral horizons, suggesting these metals to be bonded to the organic matter in a chelate form (*Bini & Michelutti, 1997*). Hg (mean 0.2 mg/Kg) presents low and uniform concentration along the soil profile.

DTPA extractable elements in the same soils present significant reduction of the metal concentration with respect to the total amount, as expected: mean extractable Cu was 5mg/Kg, Zn 1.4 mg/Kg (very low concentration, possible deficiency in plants), Pb 2.5 mg/Kg, Cd 0.1 mg/Kg, Ni 1.0 mg/Kg, Cr 0.2 mg/Kg.

It is very likely that zinc and copper pollution of surface horizons could be a consequence of agricultural practices (*Deluisa et al.*, 1996). On the contrary, As, Ni and Cr could be accumulated in the soil as a consequence of natural enrichment following weathering of parent material. However, arsenic could represent an environmental problem, since its levels in soils overcome the target value, and the limit indicated by the recent Italian law 22/97 on soil remediation (10mg/Kg).

The statistical analysis evidenced good correlation of the metal concentration with individual soil properties (e. g. Zn and Cu to organic matter and clay) and with clusters of soil samples having the same origin.

4. Conclusions

The level of contamination of the soils investigated is generally low. The soils present contents globally less than those indicated in the Italian laws concerning the trace element levels in wastes and sewage sludge to be spread on soil. Exceptions to this trend is given by anthropogenic Cu and Zn at some agricultural sites, and by natural enrichment of As, Ni, Cr.

The regional soil survey is in progress. The utilisation of a Geographic Information System allows delineation of areas with different metal concentrations, thus contributing to evaluate the soil vulnerability and the environmental hazard in response to application of fertilisers and sludge.

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DETERMINATION OF THE PEDO-GEOCHEMICAL BACKGROUND PRACTICAL APPLICATIONS

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1. Introduction

In soils, total concentrations of potentially toxic trace elements (PTTE) are easy to obtain and to interpret (provided that the reagents used can dissolve all minerals, including silicates). Most of the national regulations are still using total content values. Unfortunately, they do not supply useful information relative to mobility and bio-availability, thus to the risks for human health and the environment. On the other hand, total contents allow us to quantify stock levels which can be monitored in the course of time (Martin et al. 1998) or compared with unpolluted sites. In order to interpret the total contents, i.e. to distinguish between geogenic and anthropogenic sources, and therefore assess the level of soil contamination, the determination of the local pedo-geochemical backgrounds (PGBs) is needed.

The local PGB is the concentration of a substance in a soil horizon, only resulting from the natural geological and pedological evolutions, without any supply from human origin. This concentration varies largely from one spot to another, depending on the nature of the underlying parent material (inheritance), on the soil forming processes and their duration. Nevertheless, no place can be considered as totally free from at least slight anthropogenic air-borne depositions.

The accurate assessment of the PGB makes possible two different approaches, both using a process of type-based spatial extrapolation. The latter is based on the hypothesis that **if** certain territory has exactly the same parent material (origin, nature, state); the same "history" (pedological evolution); a similar position in the landscape and consequently shows the same sola, **then** it is highly probable that soils of this territory will present the same components, the same behaviour, the same abilities and constraints to such or such land use.

Applied to PTTE concentrations, this approach allows an expert evaluation, on any spot, of soil contamination, even moderate (Baize, 1997b, 1997c). This process of extrapolation can also be applied in order to produce a predictive map of PTTE by thematizing a soil map at medium scale (1/50.000) previously established according to a "type-based" method (Baize, 1992). That means that the major soil properties are taken into account, as far as possible (texture, sequences and thickness of horizons, structure water regime, lithology and state of weathering of the underlying rocks, etc), rather than taxonomic considerations.

This approach was first tested and applied to the Avallon area (south of the Paris basin - France) (Baize & Roddier, 1998).

2. Materials and Methods

The studied area (45.000 ha) intersects three contrasting natural regions : a granitic and metamorphic uplifted block : the *Morvan*; a liassic depression : the *Terre Plaine* and the limestone plateaux of *Lower Burgundy*. The aim was to determine local PGBs, while both reducing analytical costs and ensuring an optimal representativeness of samples.

2.1. *A sampling scheme stratified by "pedo-geochemical families"*. It has been shown that several soil series can be gathered in only one "pedo-geochemical family" (PGF) because they have undergone the same pedogenesis acting on the same parent material and therefore they have been affected by the same processes of concentration or redistribution working from a similar

geochemical inherited pool. Every PGF can be described by rather narrow ranges in the different PTTE. In addition, in the case of strongly differentiated soils (as Luvisols, Albeluvisols, Planosols, and Podzols, WRB, 1998), data must be processed distinguishing between the humus-bearing surface horizons and other horizons containing very little organic matter. Among the latter, it can be useful to discriminate between eluvial E horizons and others (Baize, 1997b).

2.2. *The choice of sampling spots* has to be directed towards the sites less affected by human activity, hence the preference given to forested sola. Of course, cultivated soils must be sampled too. Surface horizons are taken but also deeper or very deep horizons. It is advisable to have at least 25 samples available for every PGF studied. Digestion before analyses must be made using $HF + HClO_4$. Iron, as an excellent reference element (Baize, 1997a), must be determined too.

395 samples were analysed (total contents of Cd, Cu, Co, Cr, Ni, Pb, Zn, Fe, Mn and common soil data) belonging to 14 PGFs.

3. Results

As far as PTTE are concerned, major differences can be observed between the different PGFs. These are related to the lithology of the parent material, the soil type and the distance to the faults having caused high mineralizations. In addition, strong natural correlations exist, valid for every horizon of a solum as a whole. Most of the trace elements studied are correlated with clay and iron contents, or each other (for example copper/clay or zinc/iron or chromium/nickel). These correlations are usually very strong for Cu, Ni, Zn, Co, Cr and Fe, much less good for Cd and Pb.

A map was designed for every studied element with a legend taking into account a range of values (from the 1st to the 9th deciles) and not only the mean value.

4. Conclusions

Such a typological and pedological approach is not expensive because it is not systematic but analogical. So, it does not require a high sampling density. If one wishes to extrapolate the knowledge already acquired on a territory to another much larger area comprising the same PGFs, in theory no further sampling is needed. Nevertheless, some tens of additionnal samples seem to be necessary, for validation.

This is more efficient than classical methods employed by geologists (stream sediment and overbank sediment analyses) for it fits much better with soil survey. This kind of cartography should be used to design an overall soil geochemical map of France.

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COMPLEX SOIL EVOLUTION AND TRACE METALS: THE CASE OF HG IN THE ATLANTIC RANKER

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1. Introduction

Metals have been used in Pedology to shed light into soil profile discontinuities associated to erosion/sedimentation (E/S) processes in policyclic soils. Elements such as Ti and Zr are within the most frequently used because of their conservative behaviour not affected by the degree of soil evolution. The atlantic ranker is a type of complex soil formed by episodic additions of eroded material from the slopes (GUITIÁN and CARBALLAS, 1968). Between E/S episodes the soil has a stable surface which is colonized by vegetation and subjected to an enrichment in trace elements by different mechanisms as described by ROSE et al. (1979). Among them biocycling and atmospheric deposition (dry and wet) are the most important. In acid soils a great proportion of the deposited metals is retained by organic matter. So, in principle, buried surfaces of the atlantic ranker may present relative enrichments of trace elements.

We have measured the vertical distribution of Hg in an atlantic ranker trying to test its potential to detect buried surfaces and get information on its evolution. Mercury was chosen because its concentration in the soil parent material is very low (1.5 ng g^{-1}), thus the atmosphere is the main source of this element and the expected disymetry in concentration between superficial and subsuperficial horizons large enough.

2. Materials and Methods

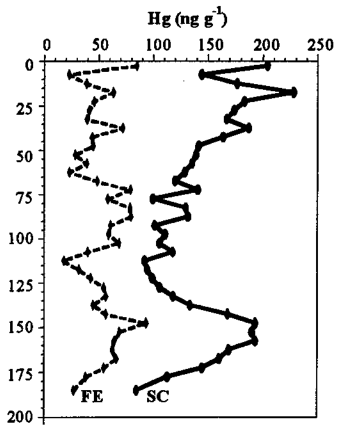
The soil sampled is 2 m deep and samples were taken at regular intervals of 5 cm. The silt and clay fractions (SC) were separated by shaking 100 g of fine earth (FE) in ultrapure water for 24 hours and later filtration through 50 µm. Mercury was measured in the fine earth and SC using a LECO-ALTEC AMA-254 mercury analyzer. For each sample triplicates were measured. Standard reference materials (SMRs) were run within each set of analysis. All the determined values were in the precision range for each SMRs and mean reproducibility for triplicates was 5.2%. Selected soil layers were radiocarbon dated indicating that organic matter accumulation began at least some 7,780 years BP ago (6,640 cal BC).

3. Results and Discusion

Figure 1 shows profiles of Hg in FE and SC. Values for Hg_{FE} ranged between 18 ng g^{-1} and 94 ng g^{-1} while for Hg_{SC} values were between 92 ng g^{-1} and 228 ng g^{-1}, thus concentrations in the SC fraction were 2.5 to 5 times greater than in FE. The vertical pattern is very similar for both fractions though more pronounced in SC. Peaks were found at the present surface of the soil, at 15-20 cm, 40-45 cm and the most conspicuous one between 145 and 160 cm. Below 20 cm the Hg content regularly decreases to minimum values of 90-100 ng g^{-1} at 115 cm. From that depth downwards, Hg steady increases until 190 ng g^{-1} and then decreases again to the bottom of the

profile.These results suggest that the ranker is composed of buried surfaces. Grain size distribution also points to that direction (data not shown). Mercury concentrations in the SC were found to be significantly correlated to the C/N ratio of the organic matter: the higher the C/N ratio the lower the Hg content. Various authors have stated that Hg in soils is strongly bound to organic compounds (BABCAN & SEVC, 1994; YIN et al., 1996), and our results also indicate that the quality of the organic matter is one of the main factors involved in the retention of Hg by the soil. For all samples the C/N ratio explains some 50% of the variation in Hg concentrations. But three populations were identified in the data: two correlated and one independent of the C/N ratio. The regression lines of the former are almost parallel, one of them containing the sample of the present soil surface and all the subsuperficial peaks. The uncorrelated samples include the one taken at the base of the profile (a CB horizon). Mercury concentrations in SC may indicate that the peaks belong to present and past soil surfaces (true stable surfaces of the ranker between E/S episodes or even A-horizon soil material eroded from the slope and sedimented in lower positions of the landscape), while low concentrations belong to samples from B or C horizons or are the result of a mixture of these with A-horizon soil material.

Figure 1. Vertical distribution of Hg. Fine earth (FE) and silt+clay (SC) fractions.



4. Conclusions

As it was expected, the atlantic ranker contained subsuperficial layers enriched in Hg. The vertical pattern and the correlation between Hg concentration and the quality of the organic matter (i.e. C/N ratio), as well as grain size distribution, all indicate that at least three buried surfaces are present in this soil. The sequence of Hg distribution from the base of the profile suggests that the ranker began its formation simultaneous to erosion in the slope soils (note the inversion in the Hg pattern at 160 cm) by accumulation of soil material over a previous down-slope soil (radiocarbon age 6,050 BP, 4,935 cal BC). Erosion probably continued until complete destruction of slope soils as the Hg contents in the middle of the profile are closer to that at the base (CB horizon). This processes seem to have been recurrent in time, with the latest erosion episode ocurring shortly after 550 years BP (1,410 cal AD).

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SPATIAL VARIABILITY OF HG AND WATER-SOLUBLE HG SPECIES CONTENT IN THE FOREST LITTER OF PODZOLIC SOILS CAUSED BY NATURAL FACTORS

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1. Introduction

Hg is known for its significant spatial variability in surface environment (RASMUSSEN, 1994). The estimation of background variability of Hg content in soils is of great importance for the best understanding of Hg behavior in soils and for a clear definition of zones with anomalous Hg concentrations. Variability of chemical composition, specifically Hg content, is most pronounced in forest soils, especially in their upper horizons. Among the factors responsible for this variability, temperature and moisture conditions, nutrients supply, type of vegetation, micro-relief, seem to be the most important (BOGATYREV, 1990).

The aim of the present work was to estimate the natural spatial variability of Hg and its water-soluble species in the forest litter and to study the causes for this variability. The causes for variability we regarded the following: 1) location of the soil profile in the catchment area (factor A); 2) degree of decomposition of the litter (factor B); 3) spatial heterogeneity of the litter horizons (factor C); and 4) random effects (factor D).

2. Materials and Methods

As the object of investigation we took the podzolic soils of the Central Forest Biosphere Reserve. The 3 studied profiles were located in the recharge and discharge areas of the river Mezha catchment. Within 1.5 m of each profile, 10 sampling points were chosen, from which we collected the samples of the L, F, and H litter horizons, characterized by different degree of decomposition of plant residues. Determination of the total content of Hg and the content of its water-soluble species (extraction H_2O:soil 10:1) in the collected samples was performed in two repetitions with the help of AMA-254 analyzer. Thus, the sampling procedure was organized in such a manner that the above-mentioned factors can be ordered hierarchically in accordance with their influence on measuring Hg content. For the assessment whether the factor exerts significant influence on the Hg content and for the estimation of the contribution of the factors to the total variance of Hg, we used variance analysis of three-level hierarchical classification (RASEMANN et al., 1995).

3. Results and Discussion

As the analysis of three-level hierarchical classification indicated that the total variance of Hg content is to the main extent determined by the degree of decomposition of plant residues, we decided to study the double-level hierarchical classification and to estimate the influence of factors A, C, and D on the Hg content in each horizon separately. The average content of Hg in the horizon (M_{av}), the estimated values of the variance components (σ), and the interclass correlation coefficients (ρ, %) are presented in Tables 1 and 2.

As it can be seen from Table 1, the contribution of the location of the soil profile to the total variance of Hg content increases from L horizon to H horizon. This implies that the content of Hg in the fresh litter-fall (L) is more strongly dependent on the conditions of soil formation (type

of vegetation, aeration and moisture content) than in the totally humificated horizon (H). This is consistent with the idea that the chemical composition of humus is not dependent on conditions of soil formation (ORLOV, 1990) and may prove that Hg distribution in litter profile is a result of soil forming processes (LINDQUIST et al., 1991). At the same time heterogeneity of the litter in respect of total Hg content decreases from the L horizon to H horizon. Therefore we concluded that the processes of decomposition and humification of plant residues result in a decrease in a spatial variability of the litter in respect of Hg.

Table 1: Variance table of measuring of the total Hg content, µg/kg

Source of variation	Horizon of the litter								
	L			F			H		
	M_{av}	σ	ρ	M_{av}	σ	ρ	M_{av}	σ	ρ
Factor A	119	23.5	41.0	199	54.9	54.3	312	123.1	76.4
Factor C		26.6	53.0		49.0	43.3		61.8	19.4
Factor D		8.9	6.0		11.5	2.4		29.6	4.4
Total		36.6			74.4			140.9	

Table 2: Variance table of measuring of the water-soluble Hg species content, ng/dm^3

Source of variation	Horizon of the litter								
	L			F			H		
	M_{av}	σ	ρ	M_{av}	σ	ρ	M_{av}	σ	ρ
Factor A	233	86.2	74.7	204	119	76.0	208	79.9	72.2
Factor C		42.9	18.6		62.0	20.7		40.4	18.4
Factor D		25.8	6.7		25.0	3.3		28.8	9.4
Total		99.7			136.8			94.1	

Total variance of water-soluble Hg species content (Table 2) is generally higher than the variance of total Hg content indicating the result of intensive migration of these species. In contrast to the total Hg content which increases from L to F and H, the content of water-soluble Hg species remains practically constant. Thus, in the litter profile Hg accumulates in course of humification in the insoluble form. The contribution of soil profile location and heterogeneity of litter horizons to the total variance does not differ significantly between horizons.

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PROFILE DISTRIBUTION OF TRACE ELEMENTS IN FOREST SOILS DEVELOPED FROM GRANITE AND GNEISS

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1. Introduction

Total concentration of trace elements in soils and their distribution in soil profiles mainly depends on parent rock and the soil-forming processes. However, the natural configurations in the ecosystems are often affected by antropogenic pollution. The content of heavy metals may be treated as a good indicator of environmental changes. But there is a need to estimate reference contents (geochemical background) in soils.

It is specially difficult to determine the reference content of trace elements in the Sudety Mountains because of high diversity of geological formations, geomorfological forms and climatic conditions. At the same time, Sudety Mts. exists in a range of atmospheric pollution (power plants emission).

The aim of this work was to determine the total contents and forms of selected trace elements in profiles of Dystric Cambisols developed from granite and gneiss of the Sudety Mountains.

2. Materials and Methods

Twenty seven profiles of forest soils developed from granite and gneiss in the Sudety Mountains were investigated. Soil samples were collected from all genetic horizons (complete with forest litter). Texture, basic properties and contents of Pb, Zn, Cu, Cd, Mn (and Fe, Al) were determined in the soil samples. The total content of metals was determined with the AAS technique after digestion with 70% perchloric acid. Several forms of elements were determined by the sequential extraction method by ZEIEN AND BRÜMMER (1988).

3. Results and Discussion

The soils are characterized by a low clay content (<10%), medium to high content of silt (26-56%) and a high amount of rock particles (gravel and stones). These are acid or strongly acid soils with pH values (in 1 mol KCl) between 2,6 to 4,2 (in mineral horizons). The pH value of the soil increases with depth. Saturation with base cations was low, 8-45%.

The highest concentration of lead in studied soil profiles was in the litter horizons (47,0-307,5 ppm). Mineral horizons contained from 23,5 to 166,0 ppm Pb. The concentration of lead decreased gradually with the depth of soil profiles to the lowest content in the bedrock horizon.The main forms of lead are fractions connected with organic matter and iron oxides. The maximal contribution of these fractions in surface horizons plays a decisive role in total distribution of lead in the soil profile.

The total content of copper in soil profiles differs less than a lead. The highest concentrations were found in litter horizons (up to 49,0 ppm, average 19,4 ppm Cu), and the lowest in the middle part of the soil profiles, in cambic horizons (average 11,0 ppm Cu). In bedrock horizons Cu concentration increased to 14,2 ppm. The contribution of mobile copper and fraction connected with iron oxides increased in soil profiles with depth, which is a symptom of copper mobility in strong acid soils and „secondary" accumulation occured in bedrock horizons.

The richest areas of Zn concentration are not surface horizons (average content in litter 48,2 ppm and in A horizon 45,9 ppm Zn). Zinc increased with depth and the maximal values were found in bedrock horizons (average 80,8 ppm Zn). The contribution of mobile Zinc, as well as that occluded on iron oxides increased with depth in the soil profile with depth. A crucial factor determining the total content of Zn in the soil profile was Zn movement and accumulation due to strongly acidic conditions.

4. Conclusions

Distribution of trace elements in the profiles of strongly acidic soils of the Sudety Mts. developed from crystalline rocks. differs for several elements. Total contents of lead decreases with depth, but inversely concentration of zinc, copper, nickel and cadmium increases with depth. It seems to be a result from different mobility and affinity of metals to organic matter and iron oxides.

The content of zinc, copper and other elements (excluding lead) in the bedrock is not a reference value, since both rock weathering and translocation of metals from surface horizons (and from pollution) increases bedrock-heavy metal concentrations under strongly acidic conditions.

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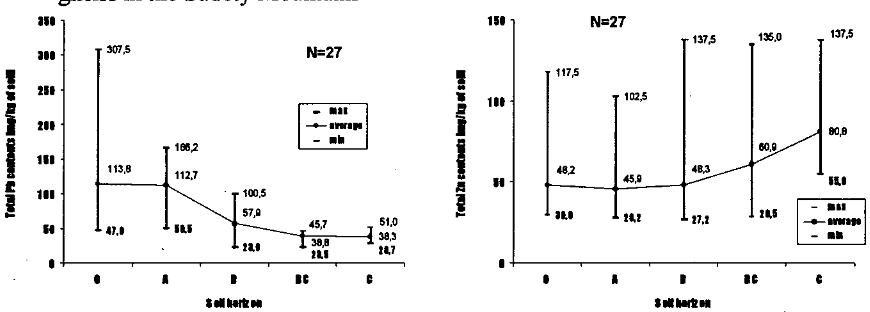
Fig. 1. Distribution of lead and zinc in profiles of Dystric Cambisols developed from granite and gneiss in the Sudety Mountains



THE DISTRIBUTION OF TRACE ELEMENTS IN SOIL PROFILE OF DIFFERENT MAJOR SOIL GROUPS OF TAIWAN

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1. Introduction

Reconnaissance and detailed survey of 0.1M HCl-extractable heavy metal contents in agricultural soils and in rice grain of Taiwan has been conducted. The differences and distribution patterns of heavy metal content of surface soil (0-20 cm) between various major soil groups in Taiwan also has been discussed (Chen and Lee, 1995). Few studies have been discussed the distribution of trace element in soil profiles of different major soil groups. The objectives of this paper are to show the distribution of trace elements in the soil profile and their relations to soil genesis of major soil groups of rural soils in Taiwan.

2. Materials and Methods

Soil samples were selected from five major soil groups in Taiwan, including red earths, sandstone and shale alluvial soils, slate alluvial soils, schist alluvial soils, and Taiwan clay. These soils can be classsified into Entisols, Inceptisols, Ultisols, and Oxisols based on *U. S. Soil Taxonomy*. Soils were sampled at the different layers, including 0-15, 15-30, 30-50, 50-70, and 70-100 cm dephts, respectively. Soil sampling was replicated three times for each layer in every soil series. The soil samples were digested with conc. H_2SO_4, HNO_3 HF, and $HClO_4$ for Cd, Cu, Ni, and Zn analysis (Page et al. (eds.), 1982), with conc. H_2SO_4, $HClO_4$, and HF for Cr analysis (Page et al (eds.), 1982), with conc. HNO_3 and $HclO_4$ for Pb analysis (Page et al. (eds.), 1982), with 9.6M HCl for As analysis (Page et al. (eds.), 1982), and with conc. H_2SO_4 and HNO_3 for Hg analysis (Page et al. (eds.), 1982). All trace elements were analyzed by flame AA spectrometer. Certified standard reference materials were used for qualified assurance.

3. Results and Discussion

Generally speaking, the amounts of trace elements are lowest in the soil profiles of sandstone and shale alluvial soils, but highest in slate and schist alluvial soils compared with other soil groups (Table 1). The distribution of trace elements may be attributed to the properties of primary parent material (Kabata-Pendias and Pendias, 1992). The contents of trace elements in soil profiles of Oxisols and Ultisols are uniform and their variation of trace elements contents in the profiles are less than those of Entisols and Inceptisols (Table 2). These results may be attributed to the short-term genesis and alluvial processes for Entisols and Inceptisols in Taiwan, especially caused by the slate and schist alluvial materials.

4. Conclusions

Different trace element contents in major soil groups in Taiwan are significantly controlled by the parent material. Soil pedogenic processes are the active force to control the solubility of

trace elements in the soils. Higher trace element contents were found in the upper parts of the profile of Inceptisols and lower trace elements contents were found in Oxisols and Ultisols. These results suggest that the concentration of heavy mentals are generally controlled by the degree of weathering processes in Taiwan.

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Table 1. The comparisons of average contents (mg/kg) of trace elements in 100 cm depth of major soil groups.

Soil Group*	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
R	10.2 [a#]	1.7 [bc]	33.3 [c]	19.9 [c]	0.14 [abc]	34.5 [d]	31.0 [b]	93.1 [d]
SS	6.96 [b]	1.5 [c]	31.6 [c]	11.9 [e]	0.39 [ab]	30.1 [d]	19.6 [c]	144 [c]
SL	9.6 [a]	2.1 [a]	46.9 [b]	23.6 [b]	0.16 [a]	51.0 [b]	54.7 [a]	199 [b]
SCH	8.8 [a]	1.7 [b]	65.6 [a]	31.9 [a]	0.10 [bc]	56.4 [a]	18.8 [c]	251 [a]
TC	9.2 [a]	1.5 [bc]	43.1 [b]	15.3 [d]	ND [c]	41.8 [c]	20.9 [c]	222 [b]

*R = red earths; SS = sandstone and shale alluvial soils; SL= slate alluvial soils; SCH = schist alluvial soils; TC = Taiwan clay.

#Same letter indicates that there is no significant difference (p=0.05) which was analyzed by SAS package (1982). ND: non-detectable

Table 2. The comparisons of average contents (mg/kg) of trace elements within Soil Order.

Soil Order*	Depth (cm)	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
Oxisols (2)	0-15	11.2	1.81	34.0	20.9	0.12	37.2	26.1	99.6
	15-30	10.8	1.90	31.8	23.2	0.12	47.8	31.5	108
	30-50	10.6	1.25	40.3	20.4	ND	33.7	29.6	92.4
	50-70	12.6	1.65	36.1	20.4	ND	36.0	26.4	115
	70-100	11.2	1.63	43.7	20.9	ND	38.4	35.0	75.9
Ultisols (2)	0-15	7.82	2.02	31.3	23.4	0.17	26.1	69.7	119
	15-30	8.83	1.87	29.3	22.4	0.16	24.4	26.5	112
	30-50	9.69	1.46	24.7	14.4	0.13	25.2	9.3	65.6
	50-70	8.95	1.74	28.4	14.2	0.08	52.6	19.9	72.6
	70-100	8.62	1.59	28.4	12.7	0.12	31.0	ND	30.1
Inceptisols (12)	0-15	8.66	2.01	44.4	22.1	0.37	45.4	39.5	212
	15-30	8.43	1.93	47.8	21.4	0.15	46.8	37.1	222
	30-50	8.86	1.93	44.8	21.8	0.11	48.2	33.5	202
	50-70	9.73	1.88	45.3	20.5	0.08	45.7	35.5	174
	70-100	10.0	1.87	47.5	21.6	0.10	46.5	28.8	184
Entisols (5)	0-15	8.93	1.52	46.7	20.2	0.23	43.6	32.9	204
	15-30	9.13	1.40	44.9	20.1	0.26	44.6	53.5	236
	30-50	7.64	1.38	44.0	19.4	0.15	42.5	28.5	196
	50-70	6.57	1.32	44.4	14.6	0.14	38.4	21.9	188
	70-100	5.23	1.12	36.7	12.7	0.09	32.3	40.2	129

*: The number in the parenthesis is the profile number of the soil groups. ND: non-detectable

Bioavailability, Fluxes and Transfer of Trace Elements in Soil and Soil Components

(Special Symposium 4)

BIOAVAILABILITY AND FATE OF TRACE ELEMENTS IN LONG-TERM, RESIDUAL-AMENDED SOIL STUDIES

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1. Introduction

The W-170 Regional Research Committee has been involved in conducting, characterizing, and evaluating the bioavailability and fate of trace elements in several long-term, residual-amendedsoils. Agronomic benefits from various inorganic and organic residuals have been recognized as providing nutrients, improving soil chemical and physical properties, and possibly have liming value. The W-170 Committee and its predecessors (W-124 and NC-118) have been actively involved in research and regulatory aspects on trace elements. As recently as 1998, a sub-committee of W-170 provided a critical peer review of a U.S. EPA risk assessment for the land application of cement kiln dust. The W-124 and W-170 committees were extensively involved in the development of the EPA 503 national sludge rule (U.S. EPA, 1993) and continue to be involved in its refinement. In addition to biosolids and manures, other residual materials include municipal solid waste (MSW), yard wastes, cement kiln dusts, pharmaceutical biomass, brewery wastes, flue-gas by-products, drinking water residualsand food wastes. This presentation will examine efforts by the W-170 group involving long-term residual trace element studies.

2. Results and Discussion

W-170 research has been used to develop and validate the predictive capabilities of trace element bioavailability, management of soils receiving short- and long-term waste amendments, and remediation of contaminated environments. Results of these studies continue to demonstrate that most organic waste products can be beneficially reused if properly managed. Monitoring plant uptake of trace elements from soils previously treated with high trace element biosolids for over 20 years indicates metal-uptake by plants on residual sludge-amended plots is minimal.

It has been well established that the bioavailability of metals in soils is higher when the source of the metals is metal salts compared to metals in biosolids. This „protective“ effect of the biosolids was factored into the risk assessment performed for the U.S. EPA 503 national sludge rule. The rule provided limits for the concentrations of 10 trace elements (As, Cd, Cu, Cr, Hg, Mo, Ni, Se, Pb, and Zn) in biosolids, and limits on the annual and cumulative loadings of the trace elements to soils. An extensive data base was utilized for the trace elements Cd, Cu, Cr, Ni, Pb, and Zn in biosolids-amended soils, but limiting data exist for As, Hg, Mo, and Se. For example, the original 503 rule provided a cumulative load limit for Mo of only 18 kg/ha, based on limited data, which would have made Mo a very restricting element for land application programs. Conversely, there is a legitimate concern about Mo-induced Cu deficiency (molybdenosis) in livestock that could develop if the Mo limit were not restrictive enough. To set a limit that is sufficiently protective without being unnecessarily restrictive will require a data set that encompasses a wide range of soil and climatic conditions. The only study that has significantly added to this data base since the 503 rule was written is being conducted by a member of W-170 (Nguyen and O'Connor, 1997).

A recent paper, however, challenged the EPA 503 rule by hypothesizing that the protective effect of the biosolids diminished with time as soil organic C oxidized (McBride, 1995). Studies by W-170 members suggests there is no increase in plant available metals over times of 15 to 20 years (Chang et al., 1997; Brown et al., 1998). For example, a study of Brown et al. (1998) demonstrated that the C losses that occurred with time after biosolids applications ceased did not correspond to increases in Cd bioavailability, and they hypothesized that the protective effect of the biosolids was at least partly due to inorganic constituents in the biosolids and not entirely to organic C. Their results suggest long-term field studies with biosolids are extremely important, and that additional research is needed to address issues that exist for all types of residuals.

Current objectives of the W-170 group that will contribute to a greater understanding of trace element chemistry and fate include: 1) characterization of the chemical and physical properties of residuals and residual-amended soils; 2) evaluation of methods for determining the bioavailability of trace elements in residuals, and; 3) prediction of the long-term bioavailability of trace elements in residual-amended soils.

3. Conclusions

Over the past decade, W-170 has been involved in studies emphasizing trace element chemistry of soils and residuals, prediction of plant uptake and movement of trace elements, evaluation of trace element chemistries in contaminated soils, and examination of the effects of soil remediation on trace element chemistry, bioavailability and mobility. W-170 research has enhanced our understanding of trace element release mechanisms, model parameters for predicting soil trace element status, crop systems and climate conditions, and land loading rates. Scientific-based findings from the W-170 group are essential for regulatory guidelines to manage the beneficial uses of residual products in a sustainable manner consistent with protecting our environment.

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MOBILITY AND BIOAVAILABILITY OF TRACE ELEMENTS IN THE SOILS OF THE CZECH REPUBLIC

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1. Introduction

Critical limits of soil pollution must refer to the defined transfer (into organisms) and transport (into water resources) pathways. Critical values of soil pollution of trace elements (TEs) for the pathway soil-fodder plants and soil-fodder plants-animals can be derived from data, characterizing not only contents, but mainly their mobilities in soils and their uptake by plants, leading to critical plant loads from the viewpoint of zootoxicity and phytotoxicity.

2. Materials and Methods

Comprehensive methods of TEs speciation in soils - sequential analysis (by Zeilen and Brümmer 1989) and simplified procedures for the determination of mobile (1M NH_4NO_3; 0.01M $CaCl_2$) and potentially mobilizable species (0.025M Na_2EDTA) were used. Total content of TEs in soils was determined in soils after destruction in HF + $HClO_4$ + HNO_3 and in plants after destruction in $HClO_4$ + HNO_3. Pot experiments were carried out with samples from 54 representative soil units (4 fold repetition). Soils used were contaminated under field conditions by airborne (emissions) and fluvial (wastewaters) inputs. The investigated set also includes soils with mainly geogenic concentration. The whole set comprises the representative range of properties of Czech soils. Responses in concentration of plants grown in these soils (rye, radish, spinach) exceed at least in maximum values critical loads in plants from the viewpoint of zootoxicity and phytotoxicity.

The obtained results were processed by elementary statistics, factor analysis, multiple regression analysis and by assessment of extreme values.

3. Results and Discussion

Previous experience based on studies of soil TEs mobilities and TEs uptakes from soils after simulated gradated pollution (salts) showed that these results can be interpreted for vulnerability assessments. This soil quality reflects the buffering potential against soil pollution.

The speciation studies of soil samples contaminated in field conditions performed by means of sequential analysis and simplified procedure revealed elements-specific and soil-specific (parent materials-specific) features of TEs bonds (Podlešáková and N□me□ek 1998). Three groups of TEs could be set up: ▪ Mn, Cd, Co, Zn and Ni with a high effective and potential mobility, pH-dependent bioavailability and transfer factors > 0.01, ▪ Pb, Cu with a high potential mobility, ▪ Be, As, Cr, V with low potential and effective mobility and transfer factors (< 0.01).

The factor analysis of the broad and the narrow standardised set of characteristics supports the finding that TEs can be subdivided into the following groups: ➻ Mn, Cd, Co, Zn, Ni (Pb, Be), ➻ the other TEs. The first factor load correlates within the first group with TEs mobile species, their plant uptakes and influence of pH. The second or third factor load correlates with TEs pool

- with total content and the potentially mobilizable content. Because the loads of the first and the second (third) factor comprise 75-65% of the total variability, they can be used for the prediction both of mobile species and mainly for the prediction of TEs contents in plants grown in this soils. We give examples of the prediction possibilities of TEs mobilities in soils and TEs uptakes (tab. 1) by plants for Mn, Cd, Zn, Ni. Substitution for TEs critical loads of plants in the prediction equation can be used for normsetting.

Table 1 Prediction equations of trace elements uptake by radish (R)

Mn:	$\ln R = 0.341 \ln MN + 0.445 \ln TO + 1.610$
Cd:	$\ln R = 0.460 \ln MN - 0.212 \ln ED + 0.952$
Zn:	$\ln R = 0.108 \ln MN + 0.209 \ln TO + 2.567$
Ni:	$\ln R = 0.265 \ln MN + 0.155 \ln ED + 0.090$

MN ... mobile species (extractable in 1M MH_4NO_3)
TO ... total content ($HClO_4 + HF + HNO_3$)
ED ... mobilizable species (extractable in 0.025 Na_2EDTA)

The behaviour of the other elements is more complicated and will be discussed in other contributions. Studies of extremes of these elements especially of As, Cu, Cr provide information concerning total contents and mobile species contents that do not cause loads of fodder plants, exceeding critical plant limits.

All obtained results provide a basis for normsetting (Podlešáková and N□me□ek 1996).

4. Conclusions

1. Mobility of trace elements (TEs) is element- and soil - specific
2. Critical soil loads for food chain protection (zootoxicity) and phytotoxicity can be derived for Mn, Cd, Zn, Co, Ni (Pb, Be) on the basis of matching critical plant loads with TEs contents and mobilities in soils leading to plant pollution
3. Critical soil loads for less mobile elements can be also derived on the basis of studies of the influence of extreme soil loads on plant uptake

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SEQUENTIAL EXTRACTION OF METALS FROM ARTIFICIALLY CONTAMINATED SOILS IN THE PRESENCE OF VARIOUS COMPOSTS

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1. Introduction

The presence of organic matter is known to affect metal solubility in soils (FÖRSTNER and WITTMANN, 1983). Some changes in metal solubility by addition of organic residues to soils have also been observed by various authors (reviewed by MADRID, 1998). The use of sequential extraction protocols, which split the soil metals into several "operationally defined" fractions, can give some useful information in order to interpret the observed effects of the presence of such organic residues (HOODA and ALLOWAY, 1994).

2. Materials and Methods

Artificially metal-polluted soil samples were prepared by adding a solution containing Zn, Cu, and Pb in NO_3 form to 300-g portions of a sandy soil in plastic containers. The amount of each metal corresponded to the maximum permitted contents in agricultural soils treated with sewage sludge. The samples were mixed with compost obtained from urban solid residue (USR), waste from paper industry (WPI) or waste from olive oil industry (OI), in proportions corresponding to 0, 65 000, or 200 000 kg ha^{-1}. The samples were then subjected to wetting/drying cycles at two temperatures (25 and 35°C) during several months. Subsamples were periodically taken from each container and sequential extraction was carried out by the EU-BCR technique (URE et al., 1993). This technique gives three fractions, which represent a progressively decreasing degree of mobility: f_1 contains mainly metals in adsorbed forms or related with $CaCO_3$; f_2 corresponds to reducible species, particularly Fe and Mn oxides; and f_3 corresponds to oxidisable matter, mainly organic matter. All experiments were done in triplicates.

3. Results and Discussion

The results commented here correspond to the first 8 months of the experiment. During this time, differences among data corresponding to different sampling periods were not significant. The experiment is still in progress, so that data for longer sampling periods will be available in the future. Thus, the recent conclusions of MA and UREN (1998) and TAGAMI and UCHIDA (1998), who found an increase of the less reactive fractions of added metals as time increases, will be checked for the present experiment. Also, significant differences due to temperature were scarce. Therefore, mainly the influence of the doses of each compost, as observed in the sample taken after 8 months of reaction, will be commented upon in this text. The behaviour of the three metals added are compared with that of Mn, native metal present in the soil in significant proportion. The three composts show significant Mn contents, especially WPI, with 2750 mg kg^{-1}.

The metals present in the composts represent significant increases in the total contents of the soil/compost mixtures, especially in the case of WPI. Only Pb is present in the three composts in contents low enough as to represent negligible increases in the total content of the metal-polluted soil. In general, the sum of the three fractions (Σ) does not increase in the proportion expected from the doses of composts. In many cases Σ even decreases when the soil is mixed with the composts, as it is the case of OI for the three metals added, USR for Cu and Pb, and WPI for Pb. Σ for Mn is not affected by OI or USR, and WPI causes large increases in Σ for Zn, Cu and Mn,

but always to a lesser extent than expected from the metal contents of the composts. The behaviour of the individual fractions is summarized below.

The most mobile fraction f_1 for Zn increases by WPI addition, while OI and USR cause a slight but significant decrease. On the contrary, f_2 increases with the three composts, and f_3 shows a less definite behaviour, with somewhat higher values for any experiment at 35°C than at 25°C. Considering that f_1 is considerably higher than f_2 or f_3, it can be concluded that OI and USR tend to decrease the mobility of added Zn.

In the case of Cu, f_1 increases very little with WPI, while USR and especially OI cause a strong depletion of this fraction. On the contrary, f_2 and f_3 are significantly increased by the three composts, especially f_3 by OI. Therefore, it seems that the more mobile forms of Cu are changed to organically bound forms, particularly by OI and USR. This conclusion agrees with that of NYAMANGARA (1998), who found that most of the Cu added to soils simultaneously treated with sewage sludge and inorganic Cu is complexed by organic matter.

Pb in f_1 decreases strongly by the presence of any of the composts, especially by OI, while f_2 and f_3 increase, again especially by OI. The individual variations in each fraction are much greater than expected from the Pb contents of the composts, but they compensate for one another and the sum Σ is only slightly affected. It can be concluded that this metal loses also part of its mobility by association to the organic matter of the composts.

Mn shows little variations in any fraction due to OI or USR, while WPI increases them. Considering that WPI is the only compost with high content in Mn, probably the variations observed are only due to the Mn present in the compost itself.

4. Conclusions

Although most of the added metals is still in the most soluble fraction after the studied time of reaction, their mobility, especially that of Pb and Cu, tend to decrease by the presence of the composts probably by association with the organic matter present in them.

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INDUCED HYPERACCUMULATION: METAL MOVEMENT AND PROBLEMS

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1. Introduction

Phytoremediation technology has become firmly established in literature since the appearance of the first paper on this subject, Chaney (1983). To date, most work has focused on the natural hyperaccumulation of heavy metals. The first report of 'induced hyperaccumulation' was made by Huang *et al* (1996) with respect to the chelate-assisted uptake of Pb by *Zea mays*. To our knowledge no study has yet addressed the potential importance of the specific metal-bearing mineral phase present. Likewise, little is known about the secondary effects application of these chemicals may have in causing metal movement out of the rhizospere and into the water table.
This paper presents data on field trials conducted during 1998 in Northern France, that monitored the effect of chemical amendments on the uptake of heavy metals and the potential movement of these metals through the soil profile. In addition, this paper discusses the possible influence the chemical form of a polluting metal may have on the choice of agent necessary to successfully induce hyperaccumulation.

2. Materials and Methods

An area of agricultural land severely contaminated by Pb, Zn and Cd as air-fall pollution emanating from a Zn smelter near Lille, Northern France, was chosen for phytoremediation field trials. The selected site had a natural vegetation cover of the Zn hyperaccumulator *Cardaminopsis halleri* and the metal tolerant grass *Arrhenatherum elatius*. Replicate plots were treated with each of EDTA and citric acid at a rate of 0.5 g of chemical per kg of soil ($75 g/m^2$ surface area). Plant samples and soil cores to 60 cm were taken each week for a period of three weeks to ascertain the efficacy of the chemical in inducing metal uptake and on promoting metal movement through the soil profile.
To complement these field studies, *Brassica juncea* was grown in greenhouse pots spiked with 1% Pb as: Pb-oxide, Pb-sulphate, Pb-nitrate, Pb-acetate. Replicates were treated with EDTA, harvested and analysed to determine the relative ability of EDTA to induce Pb uptake from each of these chemical species.

3. Results and Discussion

In contrast to Huang *et al* (1996), our data show little evidence for any significant EDTA or citric acid-induced uptake in the field (Table 1). Similarly, there is no significant evidence for a change in metal distribution within the soil profile. Perdix *et al* (1997) show that the surficial Cd, Pb and Zn contamination in this area is associated primarily with the carbonate and oxide phases, not with the organic phase. The results from our pot trial (Figure 1), show that EDTA induces only a relatively small degree of hyperaccumulation directly from the oxide salt. This could better explain the apparent inability of EDTA to induce increased metal uptake in the field.

EDTA appears to be more effective in inducing hyperaccumulation where the metals have a greater solubility and are subsequently associated with the organic phase.

4. Conclusion

Induced hyperaccumulation of heavy metals is dependant upon the chemical form of the metal present. It appears that for some mineral phases the technique will not work. Induced hyperaccumulation could be a powerful environmental tool, as it enables a dramatic increase in the list of metals for which phytoextraction is potentially viable. However, a complete understanding of the interactions between different polluting metals and the chemicals used to induce uptake is necessary, to ensure the maximum benefit of this new technology.

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Table 1. Metal concentrations (dry weight) in field experimental plants 21 days after treatment. Values are geomean and std dev.

	Arrhenatherum elatius			*Cardaminopsis halleri*		
	Cd (mg/kg)	Pb (mg/kg)	Zn (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	Zn (%)
Control	6.66 (2.76)	16.69 (4.23)	552.9 (292.2)	165.2 (36.4)	44.6 (7.4)	2.39 (0.81)
citric acid	6.77 (2.34)	16.60 (1.85)	714.4 (191.6)	123.1 (7.4)	49.2 (10.3)	1.63 (0.23)
EDTA	6.81 (1.30)	17.47 (2.46)	681.1 (222.3)	172.8 (24.3)	74.7 (29.7)	2.43 (0.51)

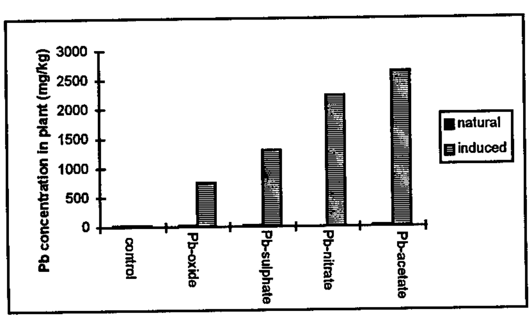

Figure 1. Natural and EDTA induced uptake of Pb by *Brassica juncea* growing in artificial 1% Pb soils.

BIOAVAILABILITY AND FLUX OF COPPER IN EDTA-TREATED SOIL

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1. Introduction

A pollutant in the soils of New Zealand is copper, because it is used at timber mill sites to prevent decay of wood. Water-borne salts of copper sulphate, as well as sodium arsenate, are common wood preservatives for fence posts. Contaminated soils have been carted away at much expense. An alternative, cheaper, and perhaps more environmentally beneficial method of remediation is phytoremediation, which is the use of higher plants to remove pollutants from the soil. Work in the United States has shown that poplar trees are effective in removing contaminants from the soil. However, no work on the use of poplar to remediate soils in New Zealand has been done. Therefore, the first objective of this research was to carry out a proof-of-concept prototype for phytoremediation of contaminated soil in New Zealand using poplar. Recently, the chelating agent, ethylenediaminetetraacetic acid (EDTA), has been added to soil to increase the solubility of trace elements for plant uptake during phytoremediation or phytomining (ROBINSON, 1997). If plant roots do not take up the solubilized element, then it will travel to groundwater and pollute it. Therefore, a second objective of this study was to determine the effect of EDTA on the movement of copper in soil with poplar.

2. Materials and Methods

A poplar (*Populus deltoides* L.) seedling was planted in September, 1997, in a large cylindrical lysimeter (85 cm diameter; 115 cm deep) with a cone-shaped bottom (46 cm diameter, base of cone) containing Manawatu fine sandy loam. On April 9, 1998 [Day of Year (DOY) 99], when the tree about 2.5 m tall, a copper nitrate solution [24.5 g $Cu(NO_3)_2.3H_2O$ in 2 L water; 3222 µg/g Cu or 6.444 g Cu total added] was applied to the surface of the soil by spraying. The Cu solution then was washed in with 250 mL water. On April 24, 1998 (DOY 114), 1 L EDTA, added at the rate of 1 g/l kg of soil was sprayed on the surface of the soil. Drainage water was collected every day between April 7, 1998 (DOY 97), and 13 May 1998 (DOY 133), the day of harvest. Twice a day, 22 L of irrigation water was applied automatically (11 L at 9:00 a.m. and 11 L at 9:00 p.m.) by 11 drippers about 10 cm above the soil surface. At harvest, the poplar tree was chopped down. All leaves were plucked off the tree, and roots were extracted at 10-cm depth increments. Soil, leaves, roots, and drainage water were analyzed for copper.

3. Results and Discussion

Table 1 shows the concentration of copper in the drainage water during the experiment.

Table 1. Copper (µg/g) in drainage water from soil with poplar.

DOY	Cu	DOY	Cu	DOY	Cu	DOY	Cu	DOY	Cu	DOY	Cu
97	0.46	103	0.36	109	0.16	115	0.11	121	0.23	128	0.76
98	0.42	104	0.06	110	0.14	116	0.13	122	0.29	129	1.25
99	0.84	105	0.20	111	0.09	117	0.13	124	0.25	130	1.83
100	0.82	106	0.24	112	0.15	118	0.13	125	0.36	131	0.15
101	0.20	107	0.21	113	0.16	119	0.14	126	0.29	132	0.84
102	0.45	108	0.26	114	0.08	120	0.07	127	0.78	133	0.63

The high values on DOY 99 and 100 probably indicate preferential flow. The EDTA added on DOY 114 mobilized the Cu, and, 13 days after the EDTA was added (on DOY 127), Cu concentrations in the drainage water started to increase and got as high as 1.83 µg/g on DOY 130. This is almost two times the drinking water standard for Cu in the USA, which is 1.0 µg/g (PUBLIC HEALTH SERVICE, 1962). Total amount of Cu lost via drainage water was 0.12 g, which was about 2% of the Cu added. The average value of Cu in drainage water without EDTA was 0.24 µg/g compared to 0.89 µg/g after DOY 127. Leaves took up negligible amounts of Cu (0.004 g Cu for all leaves), perhaps because the experiment was carried out during the autumn months in New Zealand, when plants are not vigorously growing and transpiring, as they are in the spring. Copper concentration in roots in the top 40 cm of soil was elevated (average value: 21 µg/g) compared to roots below 40 cm (average value: 9 µg/g). The concentration of Cu in the soil decreased curvilinearly with depth down to a background level of 14 µg/g, which occurred below the 80-cm depth. Concentrations of 51, 35, 28, 23, 22, 20, 18, and 16 µg/g were at the 0-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, and 70-80 cm soil depths, respectively.

4. Conclusions

The results showed that application of the chelating agent, EDTA, at the rate of 1 g per kg soil made copper more mobile and resulted in a high concentration of copper in drainage water (1.83 µg/g), which was about two times higher than the drinking water standard (1 µg/g). Future work must determine how we can use EDTA to mobilize trace elements for plant uptake, yet prevent them from moving beyond roots to groundwater.

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HEAVY METAL FLUXES IN PERTURBED SOILS AT VARIOUS MOISTURE REGIMES

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1. Introduction and Hypothesis

The risk from heavy metal inputs into a given soil as related to introduction into the food-chain and/or migration to ground water is determined by the partition of the added heavy metals between the solution and the solid-phase and further, its partition among the various components of the solid phase including clay surfaces, carbonates, iron and manganese oxides, organic matter and a residual phase consisting mostly, but not exclusively, of isomorphously substituted elements within alumosilicate minerals matrices.

We present a hypothesis that a trace constituent added to a soil in a soluble or organically-bound form will tend to redistribute between the solid-phase components (fractions) of the soil according to the initial, or prevailing, pattern of distribution ("fractionation") which is characteristic of the given soil and the given element, and which strongly depends on the soil properties. Very limited quantitative measurements of fluxes of heavy metals during redistribution among solid-phase components in soils following a pulse of pollution and the related estimates of residence time of metals in the soil solution phase, were reported. The objective of this study was to analyze a set of such measurements and analyze in detail the fluxes of heavy metals among solid-phase components and their residence time within the soil solution phase in two arid zone soils incubated at different moisture regimes for up to 1 year (Han and Banin, 1997; Han and Banin, in press).

2. Results and Discussion

Metal concentrations in the soil solution of arid-zone soils depended on the nature of metal and loading levels. In the two native arid-zone soils studied, metal concentrations were in the range Cd: 10^{-8}-10^{-9}M, Ca: 10^{-6}-10^{-7}M, Ni: 10^{-7}M, Zn and Cr: 10^{-7}-10^{-8} M. Solution concentrations of metals in the amended soils were initially increased by 2-3 orders of magnitude but rapidly (within 20-30 days) decreased back to values found in the non-amended soil. However, periodic analyses of the concentration changes in the *solid phase components* showed clearly that transfer of the metals (Cu, Cr, Ni and Zn) among them continued for much longer periods of time. The metal fluxes increased with the loading levels and were initially high and quickly decreased with time in the amended soils as illustrated for Zn in Figure 1. After about 100 days of incubation at various moisture regimes, the fluxes of metals in the metal-amended soils converged around the value of $1.0\text{x}10^{-3}$ mg $kg^{-1}h^{-1}$, with extreme values of $1.0\text{x}10^{-2}$ and $1.0\text{x}10^{-4}$ mg $kg^{-1}h^{-1}$ at the high and low loading levels, respectively. After this, the fluxes changed with time only very slightly, but remained at measurable values for the rest of the incubation period of up to 400 days. The calculated residence time of metals in the soil solution varied in the range of 1-60 h and was similar in soils incubated at both saturated and field capacity regimes. It differed somewhat among metals (Ni>Cu>Zn), and between soils (sandy>loessial), indicating that transfer of Zn among its solid-phase components was faster than that of Ni and that surface reactions were faster in the loessial soil than in the sandy soil. A somewhat striking finding was that moisture regime, when kept constant (saturation vs. field capacity) did not affect considerably the overall transfer fluxes. This implies that under the present experimental conditions, the overall metal-redistribution rate in perturbed-polluted soils kept at constant

moisture regime, is limited by the rate of the surface reactions of the metals with the solid-phase components of the soil rather than by the transport processes in the soil solution.

3. Conclusions

The rates of transformation and redistribution of heavy metals added in the soluble state to arid-zone soils are characterized by an initial fast redistribution processes, followed by a very long-term redistribution process. The overall flux of metals among fractions in the soils was the combined result of the readjustment of the metals in the native soil to changing conditions and the transfer of added soluble metals into less and less labile fractions. It can be concluded, as hypothesized above, that when polluting heavy metals are added to soils either in soluble forms or as organic sludge at low loads, they are slowly transferred and repartitioned among the solid-phase components of the soil according to the initial pattern of distribution ("fractionation") which is characteristic of the given soil and the given element, and which strongly depends on the soil properties. This conclusion is based on the present detailed study and is supported by observations and results reported in the literature for field experiments involving salts and sludge applications to humid and arid-zone soils (e.g. Mclaren and Ritchie, 1993). This finding may be of high significance in managing metal-contaminated soils and controlling and minimizing heavy metal pollution in agricultural land and in crops irrigated with reclaimed sewage effluents and amended with sewage sludge.

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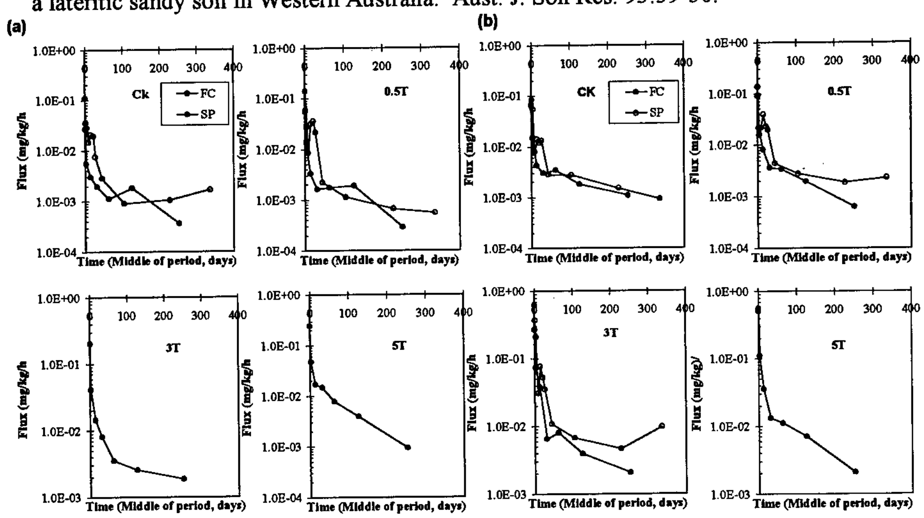

Fig. 1. Comparison of the average fluxes of Zn transfer among the solid phase components of a sandy soil (a) and a loessial soil (b) during prolonged saturated-paste (SP) and field capacity (FC) incubations. Ck - non-amended soil; 0.5T, 3T and 5T indicate levels of initial zinc salts additions, were T is the total Zn content in the non-amended soil.

FACTORS EFFECTING THE MIGRATION OF LEAD AND COPPER FROM MILITARY SMALL ARMS FIRING RANGES

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1. Introduction

The primary goal of the United States Military is to train and equip troops to maintain military readiness to defend the United States and its interests. Small arms range (SAR) training represents a major element in keeping the military ready to accomplish this mission.

Projectiles utilized as part of SAR training have accumulated over years of use. These projectiles are composed of toxic metals, such as lead and copper. These metals may pose a threat to the environment through contaminant migration.

This paper addresses factors which may effect the migration of lead and/or copper from SAR activities. A screening model to predict contaminant migration and its possible impacts on groundwater was performed.

2. Materials and Methods

Laboratory tests were conducted on soils collected from a variety of SARs to determine where metals accumulate in the soil. This was accomplished using a nested sieving technique, coupled with soil digestion and analysis, for each fraction. Several of these soils were also subjected to a sequential extraction procedure following a modified procedure as outlined by Tessier (1979). Based on information obtained from the open literature and laboratory tests, variables for a specific SAR were entered into a simple screening model, and prediction of lead's vertical migration were performed.

3. Results and Discussion

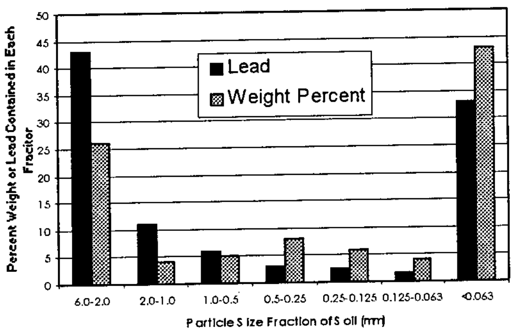
Based on open literature,a range of factors are reported to effect the transport of metals from a SAR. Laboratory results indicate that one of the main mechanisms attributing to an increased pore water concentration is the corrosion effects of the bullets as explained by Bundy (Bricka 1998). This mechanism transforms the relatively inert elemental lead into an ionic species which is available for transport.

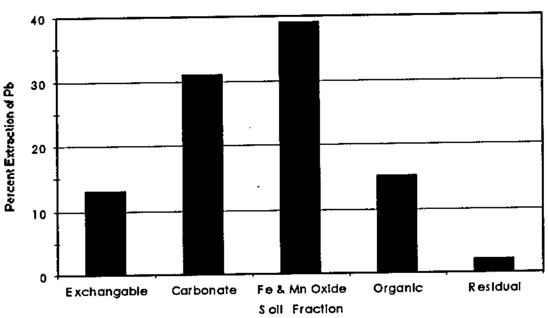
Typical sieve analysis of SAR soils tend to support the corrosion theory. As shown in Figure 1, one observes the high concentration of lead in the larger soil particles (> 2.0mm), much lower lead levels in the 2.0-0.063 mm-sized particles, and again high lead levels in the <0.063mm fraction. The high concentration of lead in the larger particles is attributed to lead particulates which was confirmed by microscopic and speciation analysis. The high concentration of lead in the small soil particles (<0.063mm) is attributed to a dissolution and re-adsorption of lead from the pore water to the soil.

Additional information is provided though sequential extraction tests. Sequential extraction analysis (Figure 2) averaged for soils collected from many SARs with varying soil type, indicates that much of the metal is in the more mobile exchangeable and carbonate fractions.

The data from the sequential extraction tests were used along with rainfall data, depth to groundwater, and the soil's cation exchange capacity in a simple modeling effort to predict the rate of lead migration for a particular SAR site. This modeling effort indicated that if

groundwater is near the ground's surface (0.75 - 1.0 meter), the vertical migration of lead may impact the groundwater in as little as five years. If the soil depth to groundwater is increased to 15 meters, this impact is not expected to be observed until 300 or more years.





4. Conclusions

Results of this investigation indicated that two mechanisms account for contaminant migration from SARs, horizonal and vertical transport. While horizonal transport, which may impact surface waters, accounts for the bulk of metals transported from SARs, the largest health risk to humans (short of soil inhalation and/or ingestion) is from vertical metals transport.

While the model results are highly site specific, the model indicates that the environmental impact of SARs on groundwater may pose a real threat to very shallow groundwater. In contrast, it is unlikely that deep aquifers will be adversely effected by metal contaminants.

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MOBILITY OF ^{109}CD AND ^{65}ZN IN SOIL INFLUENCED BY EQUILIBRATION TIME, TEMPERATURE AND ORGANIC MATTER

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1. Introduction

Evaluation of the potential mobility and biological uptake of trace metal contaminants in soil requires chemical or biological methods to quantify the fraction of soil contaminants available for biological uptake. According to Pickering (1986) multi-step extraction procedures can provide more detailed information about the status of trace metals in soils relative to single extraction methods. The fraction of metals which is readily displaced from the reversible sorption phases can be regarded as mobile phases, and can easily be distinguished from irreversible chemisorption phases (inert fractions) being released after dissolution in the later stages in the extraction scheme. Despite the significant influence temperature exerts in regulating the sorption and desorption of trace metals species in soil (Barrow, 1992), it has received little attention. Brummer et. al., (1988) reported that trace metals will after the first rapid adsorption by the soil surfaces diffuse further into sites in particles of low extractability with time. This study is therefore aimed to 1) investigate the geochemical partitioning of ^{109}Cd and ^{65}Zn with time, and 2) test and interpret the implication of organic matter and temperature for the solid phase fractions of ^{109}Cd and ^{65}Zn at increasing equilibrium time.

2. Materials and Methods

Three gram moist soil was collected from a pot experiment with temperature set to 9°, 15° and 21°C, for sequential extraction. Presented data are on oven dry soil (105°C). The soil samples were withdrawn at 0.5, 1, 3, 24, ~168 (7 days), ~720 (1 month) and ~8640 hour (1 year) after spiking with ^{109}Cd and ^{65}Zn. The sequential extractions were accomplished in the laboratory at room temperature. All extractions were carried out by shaking the samples in 50mL centrifuge tubes with 20 mL of the following extracts:

1. H_2O, pH 5.5, for 1 hour at room temperature (F1);
2. 1M NH_4Ac, pH 7, for 2 hours at room temperature (F2);
3. 1M NH_4Ac, pH 5, for 2 hours at room temperature (F3);
4. 0.04M $NH_2OH{\bullet}HCl$ in 25% HAc, for 6 hours at 80°C (F4);
5. 30% H_2O_2 at pH 2 (HNO_3), 5.5 hours at 80°C, then 3.2M NH_4Ac in 20% HNO_3 for 0.5 hour at room temperature (F5);
6. 7M HNO_3 for 6 hours at 80°C (F6);
7. Residue (F7).

Extracts and residue left after the 7M HNO_3 extraction, were analyzed for ^{109}Cd and ^{65}Zn, by a Packard Minaxi 3" through hole NaI Gamma Counter, 5000 Series.

3. Results and Discussion

The relative distribution of ^{109}Cd and ^{65}Zn at different contact times with soil is shown in Figure 1. Graphical presentations of results are made only for the 9°C and 21°C. The results indicates that soil sorption of ^{109}Cd and ^{65}Zn is rapid since less than 20-30% of ^{65}Zn and ^{109}Cd respectively were recovered in the mobile fractions (F1 to F3) after only 0.5 hour contact time. After the first rapid adsorption, a following diffusion of ^{109}Cd and ^{65}Zn towards the F5 extractable fractions, and to various degrees also towards the F4 and F6 fractions, with increasing contact time was found to be significant. Organic matter application increased the ^{109}Cd and ^{65}Zn concentrations

in the mobile fractions (F1-F3), but the effect of organic matter was also temperature dependent. For example, the concentrations of ^{109}Cd and to some degree also of ^{65}Zn in the mobile fractions were significantly increased with organic matter application at 9°C. When temperature increased to 21°C the increased was on the other hand only significant for the F3 fraction. Increasing temperature reduced the extractability of ^{109}Cd and ^{65}Zn in the mobile fractions with minor exceptions. This effect could be ascribed to the increased reaction rate between metals and soil components facilitated by increasing soil temperature. Thus, the results indicate that the organic matter induced mobilization capacity of ^{109}Cd and ^{65}Zn was reduced with increasing temperature. Although the soil sorption of the spiked ^{109}Cd and ^{65}Zn was rapid, a subsequent slow diffusion of the mobile fractions of metals towards inert soil fractions was detected (the results on reaction rates are not presented for brewery).

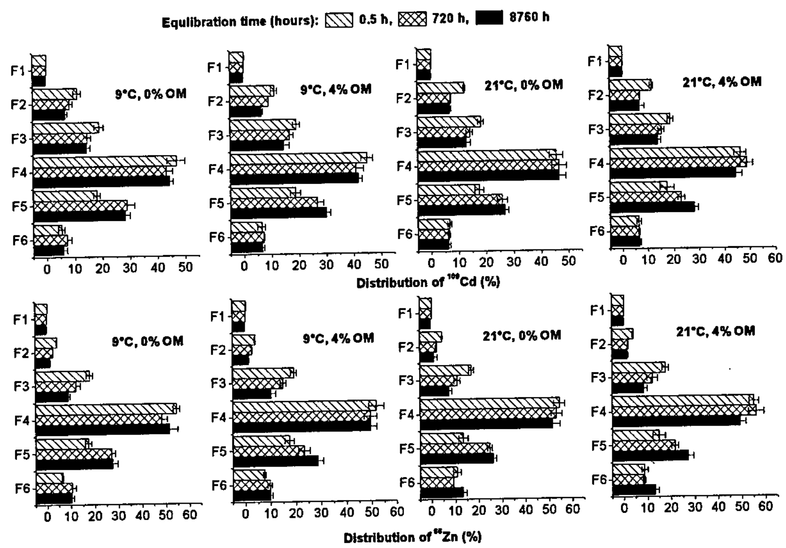

Figure 1. Relative distribution of ^{109}**Cd and** ^{65}**Zn** among the fractions in the soil **with and without organic matter addition**, at increasing equilibration time, and at **9° and 21°C**. The bars represent the mean of n=3.

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QUANTIFICATION OF BIOAVAILABILITY OF HEAVY METALS FOR PLANTS AND OLIGOCHAETE WORMS

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1. Introduction

It is the purpose of ecotoxicological risk assessment to distinguish between soils or sediments that will or will not produce effects. In the case of metals, total concentrations in soils and sediments commonly span several orders of magnitude. Organisms, however, do not respond to total concentrations, and hence soil quality criteria that are based on total concentrations are unlikely to be predictive of adverse biological effects. The total amount of a substance may not be toxicologically meaningful as it may partly be non-available for uptake by organisms. This would not be important as long as availability would be a constant factor. This, however, is not the case and it is the variation in some critical soil properties that results in a substantially different availability for uptake of compounds by organisms in different soils. This variation should be taken into account to improve the accuracy in predicting (no) effects. Especially in the case of soils there has been little consideration of the factors that modulate the bioavailability of metals. (Bio)availability needs to be dealt with as a dynamic process, comprising at least two distinct phases: a physico-chemically driven desorption process, and a physiologically driven uptake process requiring identification of specific biotic species as endpoint. It eventually is the body concentration that counts in many organisms as this is directly related with organ-effect levels. Soil organisms potentially have different uptake routes. It is thought that most organisms which live *in* the soil (i.e. including plants) are primarily exposed via pore water, organisms which live *on* the soil are exposed indirectly via their food. There is evidence for predominant pore water uptake of organic substances by soft-bodied animals, but due to their complex physico-chemical behaviour such evidence is at present only circumstantial for metals. Free metal ions in pore water are often considered to be the toxic species that can actually be taken up by organisms. Clearly, both abiotic (soil characteristics) and biotic (species-dependent) aspects determine "bioavailability".

In this contribution, results are presented of a study aimed at providing a scientific basis for inclusion of availability in assessing ecological risks of polluted sites. It is assumed that each biotic species can be considered as one of the soil phases next to the particulate phase and the liquid phase. Dynamic equilibration processes are assumed to take place between all phases present. The 'biotic phase' consists of a variety of species, each with a characteristic set of exposure routes. The equilibrium partitioning concept was used in our study as the basis for describing the physico-chemical equilibria outside the organisms. *In-situ* partitioning of 6 metals (Cd, Cr, Cu, Ni, Pb, and Zn) and As in 46 Dutch field soils was studied. In addition, a number of soil and pore water characteristics, expected to influence metal partitioning, were determined. Multivariate models were derived for predicting metal partitioning in Dutch soils, based upon easily determinable soil and pore water characteristics. The multivariate regression models are aimed to be used to predict metal pore water levels or, preferably, metal activities in the pore water on the basis of easily determinable total metal concentrations in the solid matrix and a limited number of soil characteristics. Physiologically driven uptake processes were studied for three plant species and two oligochaete worms in a sub-set of 20 of the 46 Dutch field soils sampled. Uptake and elimination of Cd, Cu, Pb and Zn was quantified as a function of time,

using compartment modelling to yield data on the uptake dynamics of the process and the equilibrium status. At equilibrium, bioaccumulation factors were calculated relative to soil sub-compartments. A bioaccumulation factor represents the ratio of the internal concentration of a chemical in an exposed biological system, to the concentration in the exposure medium.

2. Results and Discussion

Soil pH was found to be the dominant factor regulating both metal partitioning and metal uptake, and pH explained a high percentage of the variation in K_p- and BSAF-values for nearly all metals. As a typical result, Table 1 gives the multivariate regression formulae describing the quantitative relationship between K_P, and BSAF of Cd, Zn and Pb in *Eisenia andrei* or *Enchytraeus crypticus* and some soil solid phase characteristics. To further illustrate the impact of soil pH, also the formulae are given with $pH(CaCl_2)$ as the sole soil characteristic.

Table 1. Multivariate regression formulae describing the quantitative relationship between K_P and BSAF of Cd and Zn in *Eisenia andrei* or *Enchytraeus crypticus* and some soil solid phase characteristics: (1) using all solid phase characteristics, (2) using $pH(CaCl_2)$ only. Significant soil characteristics are arranged according to decreasing order of importance. Var. = variable studied; Spec.=species studied; Type = type of analysis.

Metal	Var.	Spec.	Type	Regression equation obtained	Statistics
Cd	K_P	-	(1,2)	$\log K_p = 0.28 + 0.48 * pH$	$R^2_{adj}=0.74$, n=18, F=48.4, P<0.001
	BSAF	*E. a.*	(1)	$\log BSAF = 3.19 - 0.43 * pH + 1.36 * \log clay - 1.39 * \log OM$	$R^2_{adj}=0.65$, n=18, F=11.3, P<0.001
			(2)	$\log BSAF = 2.60 - 0.30 * pH$	$R^2_{adj}=0.42$, n=18, F=13.1, P=0.002
	BSAF	*E. c.*	(1)	$\log BSAF = 1.17 - 0.92 * \log clay$	$R^2_{adj}=0.64$, n=8, F=13.2, P=0.011
			(2)	$\log BSAF = 1.87 - 0.23 * pH$	$R^2_{adj}=0.36$, n=8, F=5.0, P=0.068
Zn	K_P	-	(1,2)	$\log K_p = -0.65 + 0.61 * pH$	$R^2_{adj}=0.85$, n=20, F=111.2, P<0.001
	BSAF	*E. a.*	(1)	$\log BSAF = 3.04 - 0.39 * pH - 1.06 * \log Al_{ox} + 0.73 * \log clay$	$R^2_{adj}=0.87$, n=19, F=41.3, P<0.001
			(2)	$\log B_SAF = 2.10 - 0.39 * pH$	$R^2_{adj}=0.69$, n=19, F=40.8, P<0.001
	BSAF	*E. c.*	(1)	$\log BSAF = 3.47 - 0.46 * pH - 0.67 * \log Al_{ox}$	$R^2_{adj}=0.83$, n=17, F=40.1, P<0.001
			(2)	$\log B_SAF = 3.04 - 0.51 * pH$	$R^2_{adj}=0.75$, n=17, F=54.5, P<0.001

3. Conclusions - Evaluation

Empirical formulae as derived in this contribution do not necessarily represent true equilibration phenomena of chemicals between soil constituents and organisms; at best they reflect these phenomena. Therefore, external validation of the formulae obtained is needed, preferably using other field-soils with soil properties within the range of the properties of the 20 soils used in this study. With regard to this it should be noted that the formulae reported here, are applicable solely for field soils and for instance no predictions can be made for artificial soils that are commonly used in terrestrial ecotoxicity studies, like OECD-artificial soil. The reason for this is related to the fact that in the field soils used in this study, presumably due to natural weathering processes, equilibria between metal concentrations in the soil matrix and the pore water have established that are structurally different from conditions in artificial soils. pH is the most important parameter in this respect and the most striking consequence of weathering is the negative correlation that was found between the activity of the Cu^{2+}-ion in solution and the total copper concentration in the soils tested.

PHYTOAVAILABILITY OF CADMIUM IN SOILS AS ASSESSED BY ISOTOPIC METHODS

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1. Introduction

Methods for assessing the phytoavailable fraction of trace elements in soils are needed to evaluate the risk of transfer of metals to the food chain and to study the mechanisms that control the dynamics of phytoavailable pools. Isotopic methods have proved useful for assessing phytoavailability of P, K (Fardeau, 1981), and some trace elements as Ni and Zn (Echevarria et al., 1998; Sinaj et al., 1998). Cadmium is an element of great concern because of its toxicity and high mobility within soils and plants. This work was conducted to study the suitability and interest of the isotopic exchange kinetics methods to characterise the phytoavailable pool of Cd in soils.

2. Materials and Methods

Soil samples were collected from the Ap horizon of four brown leached soils (Luvisol, FAO) located in the vicinity of a zinc smelter in Northern France. Soils had a silt-loam texture and a pH of 8. They displayed a gradient of contamination by particulate Zn and Cd, with a total Cd content of 0.6, 8.9, 15.1 and 25.4 mg kg^{-1}. Samples were amended with a carrier free $^{109}CdCl_2$ solution, thoroughly mixed, and incubated for two days before sowing. Ryegrass (*Lolium perenne* L. cv. Prana), lettuce (*Lactuca sativa* L. cv. Reine de Mai), and the Zn- and Cd-hyperaccumulator plant, *Thlaspi caerulescens*, were grown for up to three months. After one, two and/or three months depending on the plant species, isotopic composition (IC) of Cd in shoots (i.e.(^{109}Cd/Cd in shoots)/radioactivity introduced) was measured.

In parallel, the IC of Cd in the soil solution was determined with a method adapted from Fardeau (1981) and used for Ni by Echevarria *et al.* (1998) and Shallari (1997). A soil:water system (5:10) was shaken for 18 h - sufficient time to obtain a constant Cd concentration in solution – before introduction of carrier free $^{109}Cd^{2+}$. The suspension was sampled after 1, 4, and 10 min, and radioactivity and total Cd in solution were determined after filtration at 0.025 μm porosity. Preliminary experiments had shown that the accuracy of measurements was not improved by longer exchange times. Total radioactivity introduced at time 0 was corrected for sorption of ^{109}Cd on the surface of vials and filtration apparatus. The free Cd^{2+} in the soil solution was estimated with the GEOCHEM speciation program, and IC of Cd in the soil solution was calculated (i.e.(^{109}Cd in solution /free Cd^{2+} in solution)/^{109}Cd introduced).

3. Results and Discussion

At harvest, Cd content in shoots was 0.1-2.3 mg kg^{-1} for ryegrass, 0.4-8.3 mg kg^{-1} for lettuce and 8.7-647 mg kg^{-1} for *T. caerulescens*. Isotopic compositions of Cd were similar for all plants grown on the three polluted soils. Also, they were close to the estimated IC of Cd in the soil solution. On

the non-contaminated soil (0.6 mg kg^{-1}), IC of Cd in plants exhibited unexpected large values probably because of the small concentration of Cd in soil that imposed to work close to Cd detection limits. Hence, it can reasonably be assumed that plants took up their Cd in the same pool of soil Cd, which was the isotopically exchangeable Cd.

As for other elements, e.g. P, the isotopic exchange kinetics method allowed to measure the size of the phytoavailable pools of Cd. Figure 1 shows a schematic representation of the various compartments of soil Cd according to its mobility and dynamics. The intensity factor is represented by C_{Cd}, the capacity factor by Cd_L and the quantity factor, E(t), is expressed as a function of the time considered. The exchanges between different exchangeable compartments are supposed to occur via the soil solution. This representation allows the identification of potential pools that may be accessed by plants during their growing period. Of course, plant-related parameters are not taken into account by this model.

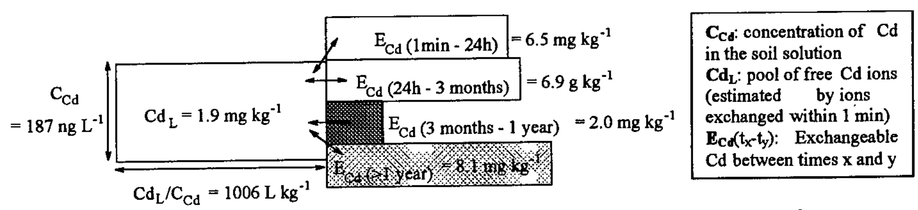

Figure 1: Cd available pools in soil with a high Cd content (25.4 mg kg^{-1})

4. Conclusions

This work has demonstrated that the isotopic exchange kinetics method is suitable to characterise the phytoavailable pool of Cd in soils. A methodology, which can be run routinely, was carefully designed for Cd in agreement with the hypotheses required for the use of isotopic exchange kinetics. Plant species were chosen to display a wide range of response to soil Cd, and they all took up the metal from the same pool, i.e. the isotopically exchangeable soil Cd. In the four soils, exhibiting high pH values, Cd was present mostly as easily exchangeable forms. Work is currently underway to characterise other soil types to determine the range of validity of the method described here.

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SOLID PHASE SPECIATION OF CD, NI AND ZN IN SOME CONTAMINATED AND NON-CONTAMINATED TROPICAL SOILS

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1. Introduction

Heavy metals contamination in the terrestrial ecosystems is the result of both geochemical processes and anthropogenic activities. Geochemical forms of heavy metals in soils affect their solubilities and thus directly influence their bioavailability. The degree of association with different forms depends on soil properties such as soil pH, organic matter, redox conditions and grain size distribution. Information on metal fractionations into different phases are almost nonexistent for soils of Bangladesh. Kashem and Singh (1998) found that the total content of Cd, Cu, Ni, Pb and Zn in soils were above the natural background level in 4 industrial sites around Dhaka city. Total contents of trace metals in soils provides only limited information when considering their toxic effects. Therefore, this study was planned to investigate the solid phase speciation of Cd, Ni and Zn in contaminated and non- contaminated soils with different soil properties.

2. Materials and Methods

Twelve soil samples were collected from the surface layer (0-15 cm) at different locations in Bangladesh. Among them 1a, 2a, 3a, and 4a were collected from tannery, city sewage, pharmaceutical and paper mills contaminated areas, and 1b, 2b, 3b and 4b from non-contaminated areas (as control). Sample 5 and 6 were collected from hilly areas and 7 and 8 from basin (flood plain) areas, respectively. Total metals (Cd Ni and Zn) in the soils were determined after digestion with *aqua regia* (Narwal and Singh, 1998). Same metals were also determined in these soils by extracting with DTPA (Lindsay and Norvell, 1978). The following modified sequential extraction method of Tessier et al. (1979) was used to determine the solid phase speciation of Cd, Ni and Zn. Two gram dry soil was used for this fractionation study and the soil/solution was 1:10. F1-Deionized water shaking for 1 h at room temperature; F2- IM NH4OAc, pH 7 and F3- 1M NH_4OAc, pH 5, shaking for 2 h at room temperature; F4- 0.04M $NH_2OH.HCl$ in 25% HOAc, 6 h in water bath at 80^0C with occasional agitation; F5- 30% H_2O_2, pH 2, 5.5 h in water bath at 80^0C with occasional agitation, then treated with 3.2M NH_4OAc in 20% HNO_3, shaking for 0.5 hour at room temperature; F6- 7M HNO_3, 6 h in water bath at 80^0C.

3. Results and Discussion

The total Cd, Ni and Zn in the soils ranged from 0.01-0.69, 21-52 and 32-939 mg kg^{-1}, respectively. Total contents of Ni except in 2 hilly soils and Zn in the city sewage (939 mg kg^{-1}) and pharmaceutical soils (162 mg kg^{-1}) were found above the Dutch limit. The Dutch A (VROM, 1990) reference values are 0.8, 35 and 140 for Cd, Ni and Zn mg kg^{-1} soils, respectively. The relative extractability, expressed as the ratio between DTPA and aqua regia extractable contents decreased from 29% for Cd, 2% for Ni and <5% for Zn in average of 12 soils. Sequential extraction procedures indirectly assess the potential mobility and boiavailability of metals in soils. Bioavailability of metals decreases in the order: water soluble > exchangeable > carbonate > oxides > organic > residual (Lena and Gade,1997) Distribution of Cd and Ni determined by sequential extraction are presented in Figure 1. The contaminated, control and normal soils

contained about 42%, 33% and 26% of Cd, and 4%, <1% and 1% of Ni, and 11%, 3% and 2% of Zn in mobile fractions (F1-F3). The mobile fraction of metals may be available for plant uptake. So, these results show that greater proportion of Cd is biologically active and may have detrimental effects than Zn and Ni (Narwal and Singh, 1998). The oxides fraction of Fe-Mn contained the highest amount of Cd, Ni and Zn among the immobile fractions (F4-F6). This may be due to the high stability constants of metal oxides. The distribution percentage of total Cd was in the following order: oxide > carbonate > residual > exchangeable > organic > water soluble and that for Ni and Zn in the order: residual > oxide > organic > carbonate > exchangeable > water soluble.

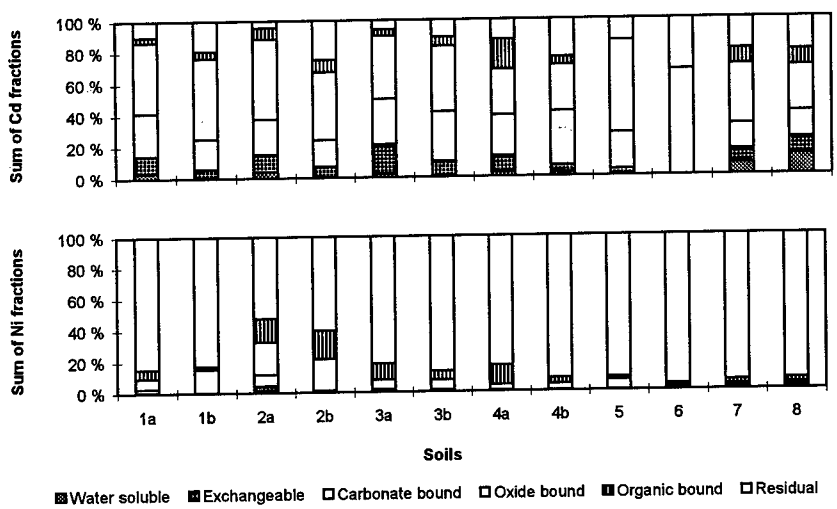

Fig1. Distribution of Cd and Ni in various fractions based on total contents in soils

4. Conclusions

Total Cd and Zn in city sewage soils were, 6 and 11 times higher than the control soils and thus they are highly contaminated. DTPA-extractant has extracted more Cd than Ni and Zn of their total contents. In average, mobile fractions for Cd, Ni and Zn in sequential extraction were 34%, 2% and 6%, respectively. For all the three metals the proportion of mobile fractions tends to increase as contamination increases due to anthropogenic activities.

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QUALITY OF ESTIMATED FREUNDLICH PARAMETERS OF CD SORPTION FROM PEDOTRANSFER FUNCTIONS

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1. Introduction

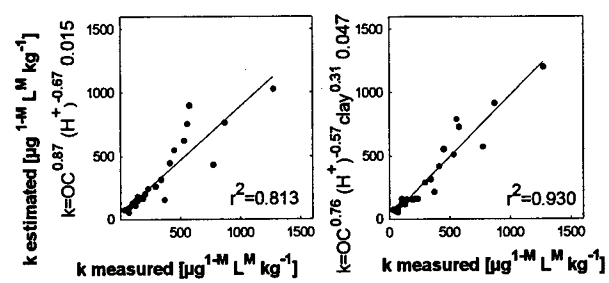
The aim was to describe Cd sorption in Ap horizons of spatially variable sandy soils in a typical catchment area ('Fuhrberger Feld', near Hannover, Germany). Therefore, the possibility is evaluated to derive a generalized Freundlich equation based on multiple regressions relating the retention parameters k and M (from laboratory isotherms using 0.01 M_c $Ca(NO_3)_2$) to basic soil properties. Such extended Freundlich equations were also used by other authors (e.g. STRECK and RICHTER, 1997). Here, the basic Freundlich term $S=k\ C^M$ (eq. 1: S: sorbed fraction, C: solution concentration) is extended to: $S = (H^+)^a\ OC^b\ clay^c \ldots k^*\ C^M$ (eq. 2). A large proportion of k is explained by H^+ activity (mol L^{-1}) and organic carbon (OC, %) and, in this study, some part by the clay (or oxide) content (%) of the soils. The remaining variability is accounted for by the residual k^* - or possibly by further variables once such are detected. Practically, the parameters k, M, a,b,c,k*... are empirically obtained from multiple linear regressions of isotherm data vs soil properties (log data). For details and the experimental procedures see SPRINGOB and BÖTTCHER (1998a,b) and caption of Fig. 2.

2. Results and Discussion

For the Freundlich parameter M (exponent), the correlation 'measured vs estimated' was weak (r^2<0.5) whereas the parameter k was well predictable by pedotransfer functions. The best regression for k was obtained if organic carbon (OC), clay content and H^+ activity were combined as predicting variables (Fig. 1b). The basic Cd sorption properties of the studied soil materials, thus, can adequately be derived by pedotransfer functions. Just the role of the variable OC remained slightly unclear as further direct multiple regressions of independently measured data of solute Cd vs soil properties yielded contradicting correlations (not shown). The measured Cd concentrations ranged between about 0.5 to 1.5 µg Cd l^{-1}. If data from fresh soil solution (centrifuging) were used for the correlation analysis, the variable 'OC' significantly increased the Cd concentration whereas the same variable acted decreasing if the Cd concentration of 0.01 M_c $Ca(NO_3)_2$ equilibrations (dried soil) was applied. It is concluded that complexing dissolved organic carbon (DOC) is involved but is more active in the fresh samples. Including 'sorbed Cd' (EDTA) or other fractions, in these regressions, never cause improvements.

Generally, the obtained k values from the isotherms were much higher than those from comparable literature studies (cited in SPRINGOB and BÖTTCHER, 1998b), probably due to lower ionic strength and absence of Cl^- in our background solutions. It is concluded that the latter two variables (possibly DOC as well) should be included if, as a further application, the calculation of solute Cd concentrations is intended. This becomes necessary for modeling Cd transport or for deriving threshold values for metal loadings of soils which ensure that critical solution concentrations will not be exceeded (INGWERSEN et al., 1998).

Figure 1: .Estimating Freundlich k from soil properties by pedotransfer functions using two (A) or three (B) basic soil properties as predicting variables



Further experiments to quantify the influence of ionic strength and Cl^- in the sandy soils of interest, especially in a very low concentration range, are currently in progress. Some results are given in Fig. 2 which show that there are really large effects. These will probably lead to pedotransfer functions comprising at least five soil and environmental variables: pH, clay (or oxides), OC, Cl^-, ionic strength. Whether such sophisticated terms - DOC and temperature are even still missing - can finally be handled in a rational way has to be discussed. However, otherwise predictions of solute Cd concentrations cannot be as correct as would be desired, meaning ± 1 µg L^{-1} or better - and modeled Cd transport in soils would hardly be reliable.

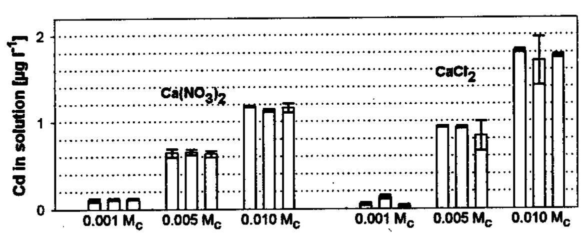


Figure 2: Influence of electrolyte composition on solute Cd in a sandy soil (3.3% C, 3.4% clay, 90% sand, pH 6, weak contamination from sewage sludge. Undried soil (10 g dry eq.) and solution (25 ml) were equilibrated at 20°C in triplicates (main bars) for 24 h and separated by centrifuging. Error bars give standard deviations of four separate measurements for each sample at a *Perkin Elmer* 1100B/HGA 700 AAS

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TRANSPORT OF TRACE METALS: LINKING THE ROLE OF COLLOIDS, REDOX CONDITIONS AND HYDROLOGY

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1. Introduction

There is an increasing awareness of non-point source pollution of waters by trace metal inputs from agricultural chemicals. Most commonly, the ability of trace metals to be dissolved and transported in drainage and runoff is evaluated by determining the total concentration of dissolved species measured using classical membrane filters of 0.22µm or 0.45µm pore size. However, recent studies have demonstrated that this so-called "dissolved form" corresponds in fact to a mixture of "colloidal" (organic and/or mineral micro particles) and "true" dissolved species (e.g. Viers et al., 1997). Until now, very few attempts have been made to characterize the composition and transport ability of colloids in shallow groundwaters. Moreover, the nature of physico-chemical parameters that control the composition and spatial distribution of colloids at the catchment scale is still poorly understood. The present work attempts to provide these data, in particular for Cu, Zn, REE, Cd, Fe, Pb, Al and Mn on the well studied agricultural catchment of Naizin-Kervidy (France).

2. Materials and Methods

The studied catchment, about 5 km^2 in surface, is located in Central Brittany. The climate is humid temperate with a mean annual rainfall of about 720 mm. The bedrock is Upper Proterozoic schist. The soil distribution and the hydrology of the catchment can be summarized by the contrast of two domains (Durand and Torres, 1995). (1) A hillslope domain comprising well drained soils where infiltration is dominant. In these zones, the water table is generally 1 to 5 m deep depending on the position along the slope. Groundwater in this domain is clear, sligthly acidic (pH between 5.5 and 6.5), always oxidized (Eh>400mV) and contains very little or no organic matter (DOC<1mg/l). (2) The bottomland, by contrast, comprises waterlogged soils. These wetlands are mostly located near the channel network. In these zones, the water table usually reaches the soil surface. Shallow groundwaters from bottomland domains are organic-rich (DOC>10 mg/l) and distinguish themselves from hillslope groundwater by a slightly higher pH (ca. 6.5). Moreover, bottomland waters exhibit temporal variations of redox potential, from ca. 400 mV in autumn to 250 mV or less in late winter and spring. Water samples from piezometers dug in the two domains and from the brook at the outlet of the catchment were collected fornightly during the autumn-spring 1997 and 1998. Temperature, pH, electrical conductivity and Eh were measured directly on the field. The collected waters were immediately filtered on site through 0.22µm Millipore membranes and acidified to pH=1. The separation of "colloïdal" particles from "true" dissolved species were performed using a tangential filtration unit, with decreasing cutoffs at 100 KD, 30 KD and 5KD (Table 1). Trace metal concentrations were determined at the Université of Toulouse (France) by ICP-MS.

3. Results and Discussion

Four important results stem from this study. (1) Organic-rich waters from bottomland areas present trace metal concentrations systematically higher than those of clear waters from hillslope domains (Table 1). This is particularly true for Fe, Cu and the REE whose concentrations in bottomland waters (Fe=25 to 1700 ppb; Cu=1 to 10 ppb; REE=1.5 to 21ppb) are higher by a factor of 10 or more than those measured in hillslope waters. This difference in trace metal concentrations between bottomland and hillslope waters is not affected by storm events and appears thus to be a long term, stationary feature of the catchment. (2) All trace metals are affected by filtration through progressively smaller pore size filters (Table 1). This result shows that trace metals are not carried in a true dissolved form in these waters, being instead strongly controlled by a colloidal fraction. However, the relative amount of trace metals carried by colloids is somewhat different in the two types of water. Indeed, while this amount reaches 100% for Al, REE, Pb and Zn in bottomland waters, it is only 30 to 70% in the clear waters from the

hillslope. In addition, DOC data indicate that colloids in bottomland waters are essentially organic in composition, whereas iron oxi-hydroxides control the colloidal fraction of hillslope waters. Taken together, these results show that the ability of waters to transport trace metals in this surficial environment is intimately related to the presence of a colloidal fraction, whose nature (organic or mineral) and transport ability are spatially variable at the catchment scale.

Table 1 : Trace elements and dissolved organic carbon concentrations in the different filtrates

	GROUNDWATER FROM HILLSLOPE DOMAINS				Groundwater from bottomlands			
	<0.2μm	<100KD	<30KD	<5KD	<0.2μm	<100KD	<30KD	<5KD
Al (μg/l)	23,0	25,0	24,2	15,0	151	101	40,8	11,3
Mn	26,4	26,3	20,7	16,7	40,5	39,8	30,2	28,2
Fe	30,0	31,0	30,0	30,0	1261	703	96,0	17,0
Cu	0,70	1,40	1,40	1,20	6,30	5,20	4,10	0,00
Zn	59,0	80,0	57,0	38,0	21,0	15,0	13,0	5,0
Pb	0,350	0,351	0,340	0,210	0,765	0,420	0,074	<0.001
La	0,394	0,342	0,317	0,174	1,490	1,056	0,626	0,114
Sm	0,060	0,037	0,031	0,014	0,413	0,304	0,192	0,040
Gd	0,040	0,032	0,025	0,013	0,285	0,208	0,139	0,030
Yb	0,013	0,010	0,008	0,004	0,067	0,052	0,038	0,010
U	0,004	0,003	0,002	0,001	0,089	0,061	0,034	0,006
Th	<0.001	<0.001	<0.001	<0.001	0,131	0,091	0,043	0,003
DOC (mg/l)	0,8	0,9	0,8	-	16,3	13,0	9,5	5,5

(3) A very significant relationship is observed in bottomland waters when physico-chemical conditions change from oxidizing to reducing. Indeed, a decrease in redox potential from 400 to 250 mV was found to be accompanied by a pronounced increase in Fe (100 to 8000 ppb) and other trace metal concentrations in the <0.22μm fraction, and to be coeval with a marked increase in DOC (from 10 to about 30 mg/l). Colorimetric titration indicated that the iron released in solution during this step was all at Fe^{2+} state. From this observation, we deduce that the transition from oxidizing to reducing conditions corresponds to a dissolution step whereby soil-forming iron oxi-hydroxides are destabilized and trace metals released into the solution. During this process, organic colloids play a critical role by providing the ligands necessary to complex the trace metals which are released during the breakdown of oxi-hydroxides. Thus, a complementary relationship is revealed in which soil-forming iron oxi-hydroxides and organic colloids play the role of donor and acceptor phases, respectively, for trace metals. (4) Concentrations of trace metals and DOC at the outlet of the catchment increase with increasing discharge. This relationship is coherent with previous hydrological studies which showed that bottomland zones make up the major contribution of the stormflow (Durand and Torres, 1995).

4. Conclusions

This study shows that colloids may be major trace metal carriers in agricultural catchments. In addition, a strong linkage is revealed between the hydrology of the system (i.e. depth of the water table) and the composition (organic or mineral) and transport ability of the colloidal fraction. These results may have important consequences for models designed to estimate the amounts of trace metals transferred from terrestrial to aquatic ecosystems.

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EFFECTS OF CARBON COATINGS ON DESORPTION KINETICS OF MOLYBDENUM FROM IRON OXIDES

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1. Introduction

The availability of molybdenum (Mo), which is needed by plants to reduce nitrate, might limit capacity of spruce to use nitrate on acid forest soils (PLASS 1983). We studied Mo mobilization kinetics from iron oxides, being the most important Mo sorbents in low pH soils (GUPTA 1997). Organic anions, often dominating the forest soil solution, have been shown to strongly influence the mobility of anions (KAISER and ZECH 1998). Thus characterizing Mo plant availability, present in soils mainly as $HMoO_4^-$ and MoO_4^{2-}(LINDSAY 1974), dissolved organic carbon (DOC) has to be taken into account especially at forest sites. The objective of our study is to examine the influence of DOC on Mo mobilization.

2. Materials and Methods

We incubated two goethites of different cristallinity (specific surface: G13=13m² g^{-1}; G97=97m² g^{-1}) with Mo for 12, 24 and 48h (800mg goethite, 160ml Mo-solution with 20mg Mo dm^{-3} as Na_2MoO_4, pH 4, at 20°C) with and without DOC being sorbed to the goethite prior to incubation (G13: 9 mg C g^{-1} ; G97: 10 mg C g^{-1}). After incubation the samples were filtert (0,45μm membrane filter) and sorption of Mo and desorption of DOC was calculated by measuring Mo and DOC concentrations in the filtrate. To remove dissolved Mo the filtered iron oxides were washed with deionized water. Afterwards 160ml of deionized water were added to the iron oxide and the suspension was divided into 20ml samples. The samples were extracted with ion exchange resin (2g per 20ml suspension) for different times (0,5-48h) to measure Mo desorption kinetics. At the end of the respective extraction period the resin was separated from the goethite by sieving. The desorption of Mo from the resin was achieved by adding 20 ml of 2M HNO_3 and shaking for 3h. Molybdenum concentrations were measured by tube AAS (SpectrAA 880Z, Varian).

3. Results and Discussion

As shown earlier Mo-desorption is biphasic (LANG and KAUPENJOHANN 1998). Fast desorption is followed by only slow mobilization at longer desorption periods. The data can be described by equation (1):

$$Mo_{des}= a_0-a_0*e^{-kt} + mt^{0,5} \qquad \text{(equ. 1)}$$

with Mo_{des}: Mo desorbed at time t [mg g^{-1}]; k: rate constant of the fast reaction; m: rate constant of the slow reaction; a_0: Mo desorbable by the fast reaction. The rate constants (m) of the slow mobilization decrease with increasing incubation time. Although effects of C coating on Mo sorption are rather low, it strongly influences Mo desorption characteristics: Mo desorbability, percentage of fast desorbable Mo and the rate constants of slow Mo mobilization increase in the presence of C at all incubation times (table 1).

The results are in agreement with the concept of diffusion controlled Mo sorption and desorption. Molybdenum is sorbed to outer surfaces (fast desorption) and inner surfaces (slow desorption) of iron oxides. Mobilization of Mo from inner surfaces is controlled by diffusion processes. The rate constant of the slow reaction depends on the diffusion parameters of the iron oxide (see annotation (2), table 1). The deeper the ions penetrate into the iron oxides the smaller the pores occupied and the diffusion constants get. The diffusion constant D is only constant for a special penetration point but not throughout the iron oxide. Consequently D can be taken as a measure for penetration depth. Keeping this in mind our data point to C coatings decreasing the accessibility of inner surfaces and slow down movement of Mo into iron oxides. Reduction of the pore volume of the iron oxides after DOC treatment determined by BJH method gives further hints for this hypotheses being true.

The effects of C coatings are greater for the more cristalline goethite G13. This is especially true for the quantity of Mo being sorbed to the surface (a_0) but also for D/r^2. The pores of G13 are less accessible for DOC. So more of the organic anions remain at the surface and can interact with the Mo added (see

below). This is supported by DOC desorption, which is faster from G13 (after 48h 3,4 mg C g^{-1} desorbed) than from G97 (2,0 mg C g^{-1} desorbed). DOC molecules can better penetrate into G97. This might be the reason for the effect on D/r^2 getting more evident at greater penetration depth in contrast to G13 where D/r^2 decreases clearly already at short incubation time.

Table 1: *Parameters of Mo desorption kinetics of the different treatments for 12, 24 and 48h incubation time and the G97 low cristalline goethite and the G13 highly cristalline goethite; „+" with DOC, „-" without DOC.*

	a_0 [mg g^{-1}]	k [h^{-1}]	m [mg g^{-1} h^{-2}]	x_m (1) [mg g^{-1}]	D/r^2 (2)
G97					
12-	0,03	5,68	0,008	3,65	4,3E-07
12+	*0,06*	*3,23*	*0,009*	*3,63*	5,5E-07
24-	0,02	0,35	0,002	3,9	2,3E-08
24+	*0,05*	*1,83*	*0,009*	*3,73*	5,2E-07
48- (3)	0,01	-	0,002	3,9	2,3E-08
48+ (3)	*0,03*	-	*0,007*	*3,7*	3,2E-07
G13					
12-	0,09	1,66	0,074	2,93	5,9E-05
12+	*0,34*	*2,24*	*0,140*	*2,88*	2,6E-04
24-	0,08	0,20	0,005	3,01	2,5E-07
24+	*0,43*	*2,55*	*0,060*	*3,33*	3,7E-05
48-	0,02	0,30	0,014	3,00	1,9E-06
48+	*0,42*	*2,64*	*0,031*	*3,43*	9,3E-06

(1) Mo sorbed
(2) $3{,}14 \cdot m^2/((x_m-a_0)^2 \cdot 36)$ see Ma and Uren (1997)
(3) only $Mo_{des}=a_0+mt^{0,5}$ could be fitted to the data

Rate constants of the fast reaction are greater in +DOC treatments than in -DOC treatments at all incubation times and for both iron oxides. Different typs of bonding to outer surfaces might be the reason: Without DOC Mo is bound directly to the iron oxide surface via ligand exchange, in the presence of DOC it might be complexed by organic molcules sorbed to the oxides. However, the parameters of the fast reaction should not be overinterpreted, because in the batch experiments conducted here desorption and complex solution kinetics might be overlapped by mass transport phenomena. Therefore p-jump experiments are planed to elucidate the Mo sorption mechanism to C coated iron oxides. The results will be presented together with the incubation experiments.

4. Conclusions

Our study clearly shows that C coatings on iron oxides increase the mobilizability of Mo sorbed. The results suggest that a slow down in Mo penetration into the goethite in the presence of organic molecules might be the reason for this finding. Especially in the case of cristalline oxides, where the rather big organic molecules are preferentially sorbed at the outer surface, surface bonding of Mo via complexation by C coatings might be enhanced. To test this hypotheses more detailed examinations regarding the bonding mechanism of Mo to DOC coated iron oxides have to be conducted. The desorption experiments conducted here imply that low Mo plant availability could occur especially in illuvial horizons, poor in organic carbon and rich in poorly cristalline oxides.

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REGULARITIES OF COPPER(II) RETENTION BY CHERNOZEM, DERNOVO-PODZOLIC AND GRAY FOREST SOILS

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1. Introduction

Some general regularities of the behavior of heavy metals in soils have been extensively studied, however the exact mechanism of their retention remains unknown. The objective of this study was to evaluate the ion balance and to clarify the details of copper (II) retention in several soils.

2. Materials and Methods

The studies were carried out on the Dernovo-podzolic and Gray Forest soil samples from Moscow region and Leached Chernozem from Tula region (Russia). The soils can be classified as Eutric Podzoluvisol, Orthic Greyzem, and Luvic Chernozem, respectively (FAO-UNESCO, 1987). The samples were converted into Ca-form. Cu-retention isotherms were obtained by treating the samples with $Cu(NO_3)_2$ solutions of various concentrations at pH 4, 5 and 6 with 3 mM $Ca(NO_3)_2$ as a background electrolyte as described by PONIZOVSKY et al. (1999). The pH value was maintained constant by adding appropriate amounts of HNO_3 or KOH. We evaluated the balance between retained Cu(II) and displaced ions. Cu(II) activity in the equilibrium solution was measured with ion selective electrode. Freundlich equation $S_{Cu} = K^*(a_{Cu})^d$ was used to approximate the isotherms (S_{Cu} and a_{Cu} are amount of copper sorbed (mmol kg^{-1}) and Cu(II) activity, K and d are coefficients).

3. Results and Discussion

For all the soils at all the pH values Cu(II) retention caused an increase of both Ca^{2+} and H^+ contents in the solution . The ratio of the displaced amounts of Ca^{2+} and H^+ ions was constant for each soil and pH value, while the sum (Ca^{2+}+H^+) was close to the amount of Cu(II) retained (Table). Therefore Cu^{2+} retention is a triple cation exchange Cu^{2+}-Ca^{2+}-H^+. Freundlich coefficient d for all cases was significantly less than 1 (Table).

One can explain the observed ratios taking into account the existence of Si-O-H groups on/in the soil particles. As far as included H^+ can not be displaced by the alkali earth, alkali or NH_4^+ ions under the conditions usual for the studied soils, the amount of these groups is not estimated with the standard CEC measurements. Due to the small radius of Cu^{2+} ion it is capable to displace such H^+ ions. The values of the d parameter of Freundlich equation less than 1 are common for the retention of heavy metals, but no explanation has been found for this phenomenon.

Table 1: Estimated cation balance and Freundlich equation coefficients for Cu(II) retention

Soil	pH	Cations displaced, mol_c per mol_c of Cu^{2+} retained			lg*K*	*d*
		Ca^{2+}	H^+	Total		
Chernozem	4	0.64	0.25	0.89	-3.055	0.33
	5	0.76	0.32	0.96	-2.704	0.377
	6	0.30	0.29	1.05	0.0743	0.816
Dernovo-podzolic soil	4	0.46	0.31	0.77	0.677	0.481
	5	0.60	0.39	0.99	-0.551	0.325
	6	0.41	0.65	1.06	0.828	0.390
Gray Forest soil	4	0.47	0.21	0.68	0.279	0.610
	5	0.59	0.15	0.74	0.281	0.602
	6	0.21	0.32	0.53	0.177	0.398

4. Conclusions

The mechanism of copper (II) retention by soils essentially differs from that of the of alkali and alkali earth metals ion exchange, and must be regarded as triple Cu^{2+}-Ca^{2+}-H^+ cation exchange.

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BIOAVAILABILITY OF CU, ZN AND MN IN CONTAMINATED SOILS AND SPECIATION IN SOIL SOLUTION

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1. Introduction

Metal contamination at former mining sites may cause toxicity in vegetation being established during land reclamation. Assessment of the metal toxicity risk is usually based on the 'total' metal content of the contaminated soil, because this has been found to correlate well with the 'soluble' content or soil solution concentration, which is 'available' for plant uptake. In order to gain a better understanding of the processes involved, however, there is a need to make measurements of soil solution concentrations during plant growth experiments assessing metal phytotoxicity.

The work reported here is concerned with metal toxicity in Australian native tree species. We analysed the soil solution of metal-contaminated soils, in order to establish the critical metal concentrations in solution that cause symptoms of toxicity in native species. In an attempt to assess the relative toxicity of organically complexed metals compared to inorganic forms, metal speciation in the soil solution was also measured.

2. Materials and Methods

Copper (Cu), manganese (Mn), and zinc (Zn) were added singly as sulfate salts to a loamy topsoil (pH in water 4.8) in amounts ranging from 16 to 2000 mg/kg with the aim of producing soil solution concentrations in the range 0.1 to 4.0 mM. Lime was also added to all of the soils and to the control samples (no added metal) to give a final pH of 5.2. The soils were placed in plant pots and watered automatically. After a period of one week for equilibration, they were planted with seedlings of *Acacia*, *Eucalyptus*, *Casuarina*, and *Melaleuca*. After a growth period of eight weeks the plants were harvested. Dry weights of the shoots were obtained and the plant material was digested and analysed by ICP-AES for a range of elements (Ca, Mg, Fe, S, P, Cu, Mn, Zn).

The plant pots contained hollow fibre soil solution samplers, which allowed periodic sampling of the solution by attaching evacuated glass sampling tubes. From five to ten ml of solution could be collected in this way. Analyses of the solutions included pH and the same range of elements as for the plant material. Speciation measurements allowed the partition of the soluble metals (Cu, Mn, or Zn) between inorganic and complexed forms.

The methodology for metal speciation was based on that of Kerven *et al.* (1995), who used size exclusion chromatography to study the complexation of aluminium in acid soils. Solutions were passed through a Fractogel TSK HW-40(S) column, which has molecular size exclusion, and only minimal ion exchange, properties. Organic molecules passing through the column were separated according to molecular weight (MW), with the high MW organics and any bound metals eluting first. Low MW (< 200 D) organics and inorganic anions and cations were retarded and eluted more slowly. The presence of organic molecules in the eluate was measured using a UV detector at 254 nm. The eluate was then collected in a fraction collector. The fractions were analysed either by AAS for individual metals or by ICP-AES for all elements of interest, including carbon (Oweczkin *et al.*, 1995).

3. Results and Discussion

The treatments with copper, manganese and zinc had contrasting effects on the native species. With copper the plants all died within a week or two. Analysis of the soil solutions showed that the pH was well below the target of 5.2. This combination of low pH (~ 4) and added copper was very toxic. At pH 4 copper should be predominantly in inorganic forms, and the speciation of the solution from these treatments showed this to be the case. This will be especially true in solutions with low concentrations of complexing ligands.

Manganese treatments produced toxicity symptoms and growth reduction of increasing severity with increasing amounts of added Mn. The pH of these treatments was initially close to the target; but increased subsequently. In all cases the soil solution speciation showed that the soluble manganese was in an inorganic form. On the basis of the treatments applied, the critical addition was the one designed to produce a soil solution concentration of 0.25 mM. This actually produced a solution concentration initially of 1 - 2 mM, which declined over the course of the experiment to ~ 0.1 mM. As a first approximation, a concentration of 1 mM can be considered to be the critical concentration for manganese for these native species.

Plant growth was affected by the presence of elevated Zn concentrations in solution. *Acacia* and *Casuarina* appeared to be the most sensitive species, and *Melaleuca* showed variable sensitivity. High concentrations of the metal (~ 0.25 mM) in the soil solutions sampled towards the start of the experiment were thought to be responsible for the growth reduction, despite the relatively high pH (5.5 - 6.0) of the solutions at that time. Speciation of the solutions showed that the zinc was only present in inorganic forms.

4. Acknowledgements

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Fate of Trace Elements in the Rhizosphere

(Special Symposium 5)

SOLID PHASE SPECIATION OF METALS IN THE RHIZOSPHERE OF FOREST AND INDUSTRIAL SOILS

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1. Introduction

The soil is an heterogeneous system. Thus, the chemistry of metals may vary considerably from one microsite to another, adding to the complexity of assessing the composition of the soil component in soil-plant relationships. For example, Wilcke and Kaupenjohann (1997) showed that greater proportions of total metal were found in the bioavailable fractions at the surface than in the core of soil aggregates. Recent work on the rhizosphere, the soil microvolume surrounding living plant roots and subjected to their influence, further documented the micro-scale heterogeneity of soils. Total aluminum concentrations exhibited a strongly descending gradient from bulk soil to the root (Smith and Pooley, 1989). Marschner and Römheld (1996) demonstrated that the bioavailability of Fe, and possibly Cu, Mn and Zn, was increased in the rhizosphere through the release of strong organic chelators by roots. Work by Courchesne and Gobran (1997) indicated that oxalate extractable Al and Fe were higher in the rhizosphere than in the bulk soil.

The objective of this study is to test the hypothesis that the relative distribution of metals between chemical forms is different in the rhizosphere than in the bulk fraction of soils and, as a corrolary, that metals are accumulated preferentially in the rhizospheric materials. Therefore, the approach was to compare the solid phase speciation of metals (Al, Fe, Mn, Cu, Zn, Pb) in the bulk and rhizosphere fractions for a series of forested and industrial soils.

2. Materials and Methods

Soils were collected at eight locations in southern Québec, Canada. At each site, three replicate profiles were sampled at two depths in the mineral soil: in the upper B and, in the lower B or C horizons. The separation of soil samples into bulk and rhizosphere fractions was conducted in the field (Gobran and Clegg, 1996). All the soil adhering to the roots after they had been shaken was considered as rhizosphere soil. This corresponded to a layer of soil less than 3 mm thick that was intimately associated to root surfaces and to the attached microflora (e.g. fungal hyphae). The remaining material was regarded as the bulk soil. Chemical extractions with $BaCl_2$, Na-pyrophosphate, ammonium oxalate and HNO_3–HCl were used to operationally define the solid phase chemistry of Al, Fe, Mn, Cu, Zn and Pb in bulk and rhizosphere materials. Metals in triplicate extracts were determined by inductively coupled plasma - atomic emission spectrometry (ICP-AES). X-ray fluorescence (XRF) analyses were also performed on the bulk fraction of each horizon. Organic C content, pH in water, and the quantification of fungal communities and of bacteria completed the analytical scheme.

3. Results and Discussion.

The chemistry of the rhizosphere is enriched in organic carbon and more acidic than the surrounding bulk soil (Figure 1). Microbial analyses showed that fungi (e.g. *Aspergillus* sp.) are more abundant in the rhizosphere than in the soil matrix. Also, the rhizosphere of forest soils developed under Norway spruce (*Picea abies*) contained a significantly ($\alpha \leq 0.10$) lower amount of weatherable minerals (e.g. amphiboles) than the bulk soil. These data suggest that the acidity generated by the activity of roots and associated microorganisms creates a corrosive microenvironment enhancing mineral weathering and the release of cations. Extractions with Na-

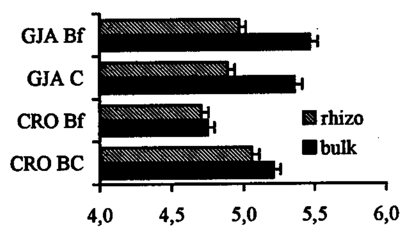

Figure 1. Soil pH in water

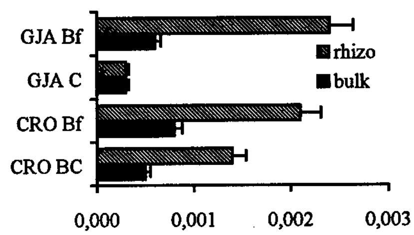

Figure 2. Zn in BaCl2 extract (g/kg)

pyrophosphate revealed that the abundance of organic substances in the rhizosphere increases the formation of Al-, Fe-, and, to a lesser extent, Cu-organic complexes close by roots. In forest soils, $BaCl_2$ -exchangeable metal contents were higher, by a factor of up to five, in the rhizosphere (Figure 2). Except for Zn, acid-extractable metals were also enriched, generally by less than 50%, in the rhizosphere of forest soils. The reverse trend was observed at industrial sites where the metal content in the rhizosphere was generally equal or lower to that in the bulk.

4. Conclusions

Our observations establish the rhizosphere as a distinct and dynamic environment within the already heterogeneous soil system and stress the importance of a proper assessment of soil micro-variability in process-oriented ecosystem studies.

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BIOAVAILABILITY OF TRACE ELEMENTS AS RELATED TO ROOT-INDUCED CHEMICAL CHANGES IN THE RHIZOSPHERE

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1. Introduction

Predicting the bioavailability of trace elements to plants is a major agricultural and environmental issue. Indeed, plants are the prime entry point for trace elements in the food chains. On the one hand, for trace elements that are essential micronutrients for plants, animals and human beings, the question arises of ensuring adequate levels in agricultural products. Indeed deficiencies of micronutrients are fairly widespread in various types of soils worldwide. On the other hand, as a consequence of human activities, concentrations of potentially toxic, trace elements tend to build up in some environments and ultimately raise the question of the contamination of the food chain. Understanding the mechanisms that are implied in the acquisition of trace elements by plant roots is thus a prerequisite for assessing their bioavailability. The aim of this paper is to give an overview of how chemical changes induced by plant root system in the so-called rhizosphere can influence the dynamics of trace elements, their speciation in the soil solid phase and their bioavailability to plants. This short review will focus on the major, direct effects of the root itself and will not address the effect of rhizosphere microorganims.

2. Root-induced changes in ionic equilibria and bioavailability of trace metals

The prime function of plant roots is the uptake of water and nutrients which results in severe changes in ionic concentrations of the soil solution in the rhizosphere. Indeed, the differential rates of uptake of water and ions contained in the soil solution causes a depletion or an accumulation of the ionic species in the rhizosphere, according to their bulk concentration. Trace elements that occur at low concentrations in the soil solution are thus likely to be depleted as a consequence of plant uptake, unless the root is very efficient at restricting their uptake (exclusion strategy). In addition, the root-induced changes in ionic concentrations of other ions, major cations such as K, Ca and Mg in particular, will shift the ionic equilibria involved in cation exchange reactions in the soil surrounding absorbing roots. These changes can have dramatic effects on the dynamics of those trace elements that occur as more or less exchangeable cations. This is examplified here in the case of ^{137}Cs and Cd.

3. Root-induced changes in pH and pe and bioavailability of trace metals

The exchange activities of plant roots also result in profound changes of pH and redox potential (pe) in the rhizosphere which both deeply affect the dynamics of trace metals, either through a direct effect on their speciation, or through a solubilisation of the trace metal bearing mineral phase. Root-induced changes in pH occur primarily as a consequence of the differential rates of uptake of cations and anions by plants, which are compensated for by a release of either protons or hydroxyl/bicarbonate ions in the rhizosphere. The subsequent acidification or alkalinisation of the rhizosphere will thereafter result in increased or decreased solubility of those many trace metals whose speciation is pH-dependent. For those trace elements which can occur under various oxidation states in the soil (such as Cr, Mn or Se for instance), changes in pe will be of prime importance. The reducing activity of the roots of many species is thus likely to alter the

speciation of those elements. In addition, in the soil, many trace metals are associated with minerals such as metal carbonates or oxides that can be solubilized under acidic or reducing conditions or can conversely precipitate under alkaline or oxic conditions. Root-induced changes of pH and pe can thereby affect the bioavailability of trace metals in the rhizosphere as will be examplified for Zn and Mn.

4. Root exudation of complexing substances and bioavailability of trace metals

Plant roots are known to release tremendous amounts of C as various types of exudates that are a prime source of energy for rhizosphere microorganisms. Some of these, in spite of contributing to only a minor proportion of root-exuded C, are very reactive substances which can sometimes exhibit strong complexation properties with respect to a whole range of trace metals. Among these are carboxylic anions such as citrate, oxalate or malate for instance. In addition, roots of graminaceous species can release the so-called phytosiderophores which can strongly complex Fe and also a range of other metals such as Cd, Cu and Zn. Several examples will be adressed that show how these various types of complexing root exudates can directly affect the bioavailability of trace metals.

5. Conclusions

Rhizosphere chemical conditions can thus be drastically different from that of the bulk soil, as a direct consequence of the activity of plant roots. The mechanisms involved in these root-induced changes and in the acquisition of trace elements can widely vary among plant species, and in response to environmental conditions too. This diversity needs to be further studied and used in the future, although it definitively complicates the reliable prediction of the bioavailability through a single, simple, universal, chemical soil test.

CAN RHIZOSPHERE CHEMICAL CHANGES ENHANCE HEAVY METAL ABSORPTION BY PLANTS GROWING IN CALCAREOUS SOIL?

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1. Introduction

Smelter emissions caused atmospheric deposition of lead (Pb), zinc (Zn), copper (Cu), cadmium (Cd), and arsenic (As) in West Texas before pollution control devices were installed. Widespread metal dissemination occurred, but nearby soils contained heavy metal concentrations >3,000 mg kg^{-1}. The effect on riparian vegetation is difficult to generalize. Plants are often classified as metal accumulators or excluders. Root absorption is by passive diffusion or active absorption., or metals can be absorbed by foliage. Alkaline (calcareous) soils were thought to be a barrier to plant accumulation of metals. Initial evaluation suggested that the food chain was vulnerable.

2. Materials and Methods

A general survey of ten perennial plant species was made from the riparian plant community of the Rio Grande River floodplain. Collections were characterized by taxonomy and chemically analyzed for Cu, Zn, Pb, Cd, Mn, Ni, Cr, Co, V, and Ca using ICP spectroscopy. River sediments were collected from the shallow banks of the river. Soil samples were collected about 1km above and 1km below a metal refinery at the surface and shallow subsurface in the flood basin and shoulder terrain of the Rio Grande River, El Paso, TX. An additional set of plant and soil samples was collected upstream at Canutillo, TX (11 km west of the refinery). All samples were collected in triplicate and processed for metal analysis.

Specific soil samples were collected in triplicate from areas with salt cedar (*Tamarix gallica*) and bermudagrass (*Cynodon dactylon*) growth. Soils samples included bulk (no plant growth), rhizosphere (soil sieved from the root mass), and soil-plant interface (soil adhering to roots). Soil samples were analyzed to characterize metal forms and the availability to plants. Soils were sequentially extracted by the traditional procedure for heavy metal characterization; exchangeable metals, carbonate-associated metals, easily reduced manganese oxides metals, amorphous iron oxides metals, sulfidic-organic metals, and residual metals.

3. Results and Discussion

Sediments and Soil Low metal concentrations were found in river sediments. Sediment loads are likely continuously moving downstream due to water flow. However, at the smelter site some elevated concentration of Cu (23.9 mg kg^{-1}) and Mn (184 mg kg^{-1}) were found, being three times the values 11 km upstream. Surface soils near the smelter showed that high concentrations of Cu, Pb, and Zn, metals commonly refined at the smelter. Slightly elevated concentrations of Cd, Ni, and Co were also found. To a lesser extent, subsurface soil also showed metal contamination that could be due to levee disturbance from road shaping. Soils upstream from the smelter showed metal concentrations within conventional ranges.

Vegetation Three perennial plant species, salt cedar, clover, and bermudagrass were common to both smelter and upstream sites. Plant tissue Cd and Pb were at least two fold greater in clover and salt cedar growing at the smelter site. Bermudagrass at the smelter site had high concentrations of Cd, Zn, Cu, Pb, and Mn in leaf blades. bermudagrass appeared to be an indiscriminate metal accumulator.

Bulk, Rhizosphere and Soil-Root Interface Soils Heavy metal concentration in the various soil fractions showed differences in heavy metal concentrations and form. Both $CaCO_3$ concentrations and metal soluble complexes changed with the presence of plants (salt cedar and bermudagrass). There appears to be less $CaCO_3$ in plant rhizospheres than in bulk samples. There also appeared to be less Pb and Cu in the more insoluble manganese oxide, amorphous iron oxide compounds. Soil Mn appeared to be more concentrated in the rhizosphere than in bulk soils in the carbonaceous associated metals and the easily reduced manganese oxides. Manganese in bulk samples was associated more with the insoluble forms of ferric oxides and sulfidic organic complexes.

4. Conclusions

In alkaline-calcareous soil, bermudagrass accumulates heavy metals. The other plant species in the survey did not accumulate excessive concentrations of metals. The soil in the root zones of bermudagrass and salt cedar was modified by the chemical action of the growing plant and other rhizosphere processes. Calcium carbonate was reduced in the root zone, which signifies a reduction in soil pH. Lead, Cu and Mn were found in greater soluble forms in comparison to bulk soil (no plant growing). There are no domesticated animals grazing in this contaminated area. However, the persistence of heavy metals likely affects riparian wildlife. Results also suggest that alkaline, calcareous soils may not be a metal barrier for grasses and entry of metals into the food chain is possible from animals feeding on grass.

HEAVY METALS AT THE SOIL-ROOT INTERFACE: INTERACTION OF CU(II), PB(II), ZN(II) AND CD(II) WITH A CA-PGA NETWORK

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1. Introduction

The transfer of nutrients through the soil-root interface and the apoplast is a fundamental step in the plant nutrition. The free-space volume is closely related to the fibrillar arrangement of the root mucilage which depends on the composition of the soil solution and on the affinity of the ions for the polymers which constitute the mucilage. Heavy metals are very dangerous as they tend to destroy the porous structure of the mucilage (GESSA and DEIANA, 1991). Here we report on the results of a survey regarding the interaction between Cu(II), Pb(II), Cd(II) and Zn(II) ions and an artificial network of Ca-polygalacturonate (Ca-PGA) with a fibrillar structure similar to that observed on the plant roots.

2. Materials and Methods

The Ca(II)-PGA networks were synthesized as previously reported (GESSA et al., 1997). The Cu(II), Pb(II)-network systems were subjected to the Scanning Electron Microscopy (SEM) and to X-ray mapping analysis. Copper(II), Pb(II), Cd and Zn(II) concentrations were quatified by an HPLC Dionex apparatus. The absorption kinetics were carried out by using a reaction volume equal to 100 ml.

3. Results and Discussion

The absorption kinetics at pH 3.5 and 5.5 of Cu(II) (600 μM) and Pb(II) (600 μM) by the Ca(II)-PGA network in the presence of calcium acetate (6.0 mM) indicate that Pb(II) has an affinity towards the functional groups of the network fibrils slightly higher than that of Cu(II), the absorption being equal to 52 and 43 μmoles / 180 μmoles of -COOH groups at pH 3.5 and 49 and 41 μmoles at pH 5.5, respectively. The absorption kinetics performed at the same pH values in the presence of both the ions (60 μmoles of Cu(II) and 60 μmoles of Pb(II)) (Fig. A) indicate that the amount of Pb(II) absorbed does not vary considerably whereas that of Cu(II) decreases to 25 and 15 μmoles at pH 3.5 and pH 5.5, respectively. This behaviour can be attributed to both hydrolysis reactions and the network structural conformation. The increase in the absorption capacity of the network from 52 (system with only Cu(II)) to 75 μmoles (system with Cu(II) and Pb(II)) at pH 3.5 and to 65 μmoles at pH 5.5 can be reasonably attributed to the higher total ionic concentration in solution (1.2 mM). The same systems studied in the presence of Cd(II) and Zn(II) ions (60 μmoles of each ion) at pH 3.5 (Fig. B) show that the addition of these ions causes a decrease in the amount of Pb(II) and Cu(II) absorbed and the whole absorption capacity markedly change at pH 3.5 (102 compared to 75 μmoles) while it remains constant at pH 5.5. Therefore, it can be hypothesized that the Cd(II) and Zn(II) ions compete with Pb(II) and Cu(II). The absorption of Cu(II) ions in the network cause the formation of microspheres, easily distinguishable in the SEM microphotographs,

with a high Cu(II) concentration as shown by the X-ray mapping. On the contrary the Pb(II) ions lead to the collapse and the destruction of the fibrillar structure of the network. The IR spectra of the Pb(II)-PGA and Cu(II)-PGA complexes suggest that the carboxylate groups coordinate the ion through a sole oxygen atom. In contrast, the spectra of the Cd(II)-PGA and Zn(II)-PGA complexes, similar to those of the Na- and Ca-polygalacturonates, indicate a bidentate structure of the carboxylate groups in these complexes. The electron spin resonance measurements at room temperature of the Cu(II)-PGA network show an anisotropic axial spectrum. The spectral parameters ($g_{||} = 2.389$, $g_{\perp} = 2.070$ and $A_{||} = 120$ G) are characteristic of a copper environment with a relatively strong axial perturbation. This evidences that the Cu(II) absorption is accompained by the formation of an inner-sphere complex. These findings suggest that a purely electrostatic interactions between the hydrated Zn (II) and Cd(II) ions and the carboxylate groups occurs whereas the Cu(II), and reasonably Pb(II), ions form inner-sphere complexes with the carboxylate groups and justifies the markedly higher affinity of such ions compared to that of Zn(II) and Cd(II). Support to such an hypothesis comes from further observations (in progress) that Cd(II) and Zn(II) ions, unlike Pb(II) and Cu(II), do not form stable complexes with monomeric uronic acid.

4. Conclusions

The results of this study provide new information regarding the effects of the Cu(II) and Pb(II) ions on the physical properties of the network. Therefore, they have a great biochemical significance connected to the transfer of these heavy metals through the soil-root interface. Covalently bound ions are very dangerous as they tend to collapse the fibrils of the root mucilage destroying its porous structure and thus hindering the flow of nutrients. Moreover, evidence is given regarding the important role of the polymeric network in the complexation of Cu(II) and Pb(II) ions acting as an accumulation phase, especially at low ionic concentration.

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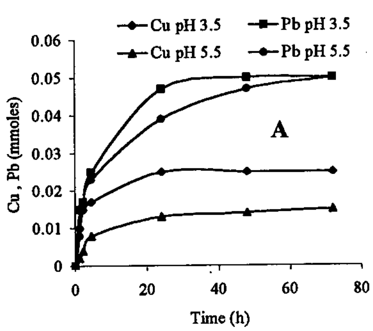

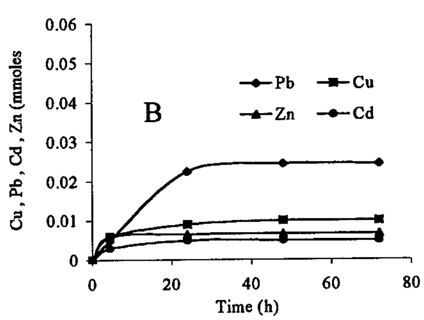

Figure 1- A: adsorption kinetics at pH 3.5 and 5.5 of Pb(II) and Cu(II) by the Ca-network in the presence of Ca-acetate 6.0 mM. The initial concentrations were Cu(II) 600 μM; Pb(II) 600 μM.**B:** adsorption kinetics at pH 5.5 of Pb(II), Cu(II), Zn(II) and Cd(II) by the Ca-network in the presence of Ca-acetate 6.0 mM. The initial concentrations were Cu(II) 600 μM; Pb(II) 600 μM; Zn(II) 600 μM; Cd (II) 600 μM.

A PHYSIOLOGICAL AND GENETIC INVESTIGATION OF THE ROLE OF ROOT EXUDATES IN ALUMINUM TOLERANCE

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1. Introduction

Acid soils limit agricultural productivity in many regions of the world, including significant areas of the United States. Aluminum (Al) toxicity is one of the major factors limiting crop production on acid soils (Von Uexküll and Mutert, 1995). Research programs representing a diverse array of scientific disciplines are addressing the issue of acid soil-related limitations to crop productivity. Included in this effort is research by plant physiologists, molecular biologists and geneticists on the basis of plant Al tolerance. To date, our understanding of the biology of plant Al tolerance is still in its infancy, and broad questions remain regarding the Al tolerance mechanisms that plants rely upon, as well as their underlying genetic and molecular basis. One of the most exciting new areas of research in this field involves a recently identified mechanism of Al exclusion involving exudation of Al-binding ligands from the root. In Al resistant wheat genotypes, Al exclusion has been shown to be localized to the root apex. Delhaize, Ryan and coworkers subsequently showed that in near isogenic Al resistant and sensitive wheat lines, this Al exclusion was due to Al-induced malate release controlled by the single, *alt1* locus (Delhaize et al, 1993; Ryan et al, 1995a.b). The Al-induced malate efflux is localized to the root apex, triggered very rapidly and specifically by Al^{3+} (and not other toxic cations) and appears to involve an Al-induced malate transporter (presumably an anion channel) in the root cell PM (Kochian, 1995). In this presentation, the state of the current research concerning the role of root exudates, with a focus on root release of organic acids, in Al tolerance in crop plants will be covered.

2. Materials and Methods

Plant materials, seedling growth and root exudate analysis. Seeds of winter wheat (*Triticum aestivum* L) cultivars (Al-resistant Atlas 66 and Al-sensitive Scout 66) and near isogenic Al-tolerant and Al-sensitive wheat lines (ET3 and ES3), as well as seeds of Al tolerant and sensitive maize genotypes (SA3 and SA5) were used for these studies. Seedlings were surface sterilized, germinated and grown in sterile culture in 125 ml flasks containing 20-60 ml of filter-sterilized solutions (control solutions: 100 μM $CaCl_2$ pH 4.5, or Al treatment solutions: 100 μM $CaCl_2$ + 5 or 20 μM $AlCl_3$, pH 4.5). The flasks containing 2-3 seeds were incubated on a shaker (132 rpm) in a growth chamber with a 20^0 C day (16h), 15^0 C night (8 h). The procedures used to collect, concentrate and analyze root exudates (using a Dionex ion chromatograph) are described elsewhere (Pellet et al, 1995; Huang et al., 1996). In experiments designed to study spatial aspects of organic acid and Pi exudation, root segments of 5d-old seedlings were excised sterilely . Both the apical segment (either 0-5 or 0-10±2 mm from the apex) and the adjacent subapical segment (either 5-10±2 or 10-20 mm from the apex) were used. Five to ten root segments were exposed to the appropriate solution for 7

hours. In experiments designed to monitor rhizosphere pH, plants were grown for 5 days in 900 ml pots filled with aerated control solution.

3. Results and Discussion

In a broad set of experiments ranging from studies at the single-celled level investigating Al regulation of citrate and malate release from root apical protoplasts, to physiological and genetic studies with intact plants, we have accumulated considerable data indicating that Al-induced organic acid release is an important Al tolerance mechanism in cereal crops. Using patch clamp techniques to study Al-induced citrate release in maize, we have characterized an Al-inducible anion channel that appears to mediate citrate release in root apical cells of the Al tolerant (SA3) but not the Al-sensitive (SA5) maize lines. A detailed examination of this channel will be presented. At the whole root level, we have investigated Al-inducible malate release in wheat as well as citrate release in maize, and results from diffusion-based modeling of these systems to determine if the concetrations of organic acid in the rhizosphere are high enough to exclude Al from the root tip will be presented.

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METAL SPECIATION IN SYMBIOTIC FUNGI (ECTOMYCORRHIZAE) USING MICRO X-RAY SPECTROSCOPY

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1. Introduction

Ectomycorrhiza is a symbiosis between fine roots from trees and soil fungal biomass, and is crucial for providing the tree with inorganic nutrients such as nitrogen, phosphorus, zinc, copper and iron (Paul and Clark, 1989). The mycorrhizas themselves are also able to accumulate considerable amounts of metals such as zinc, copper and cadmium (Berthelsen et al., 1995). Thus the mycorrhizal symbiosis may act as a filter for enhanced uptake of potentially toxic trace metals into tree tissue (Turnau et al., 1996). How these structures bind such large amounts of metals is however not well understood. The present project aimed at revealing major binding forms for Zn and Cu in various types of ectomycorrhizae, and distribution of these metals within the mycorrhizal structure.

2. Materials and Methods

X-ray data for metal speciation were collected with respect to 3 types of mycorrhizae: *Cortinarius* sp. (*in vivo*) (Zn) from an area with high Zn levels in organic surface soils, partially dried *Cenococcum graniforme* (Zn and Cu) and *Russula* sp. (Zn) from an area with low Zn and Cu levels in surface soils. XANES data (X-ray Absorption Near-Edge Spectroscopy) extending into the EXAFS region (Extended X-ray Absorption Fine Structure) were collected at the Zn and Cu k-edges on hot spots in the mycorrhizal mantle, and on Zn and Cu oxalate standards. The distribution of Zn within the mycorrhizal structure was investigated through X-ray fluorescence mapping of a thin dried *Cortinarius* sp. cross-section.

3. Results and Discussion

At present results from Zn speciation and distribution in *Cortinarius* sp. only are evaluated, showing clearly that Zn is bound as Zn-oxalate in the fungal part of the symbiosis. Fungi produce oxalate via the tricarboxylic cycle in mitochondria and glyoxylate cycle in the cytoplasm (Dutton and Evans, 1996). Oxalate is important for fungi concerning for instance fungal pathogenesis, competition, nutrient uptake and possibly detoxification of copper-containing fungicides. Since Zn and Cu-oxalate is highly insoluble, oxalate precipitation may be an important means of metal immobilization and detoxification in fungi.

Mapping of the Zn distribution in the mycorrhiza revealed that far the most Zn accumulated in mycorrhiza was located in the outer, mycorrhizal mantle. Zn levels in the interior of the mycorrhiza, including the root part of the symbiosis, were very low.

Conclusions

Oxalate produced in fungal cells precipitate Zn in the surface layer of the mycorrhiza, most likely buffering excessive Zn uptake into the tree. Further evaluation of the micro-XANES data may reveal whether such an immobilization mechanism is applicable to Cu. Oxalate precipitation may in fact be an important immobilization mechanism in fungi with respect to other, toxic trace metals.

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RHIZOSPHERIC MOBILISATION OF RADIOCESIUM IN FOREST SOILS DIFFERING IN WEATHERING STAGE

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1. Introduction

The radioactive deposition on forest canopies after the Chernobyl accident resulted in the accumulation of radiocaesium in the surface horizons of soils which are major sources for ^{137}Cs plant contamination. Large uncertainties characterize the model predictions concerning Cs contamination of above standing vegetation because transfer factors have important sources of variation. Relatively to modelling, a complementary approach could be based on the categorization of forest ecosystems. In this respect, assessing potential Cs soil-to-plant transfer requires a standardized test quantifying the Cs mobilization by plant roots from contaminated soils in controlled conditions.

2. Materials and Methods

Six forest soils have been selected in various european locations (Austria, Germany, Italy). They derive from various parent rocks and simulate a fictive "weathering" sequence eutric cambisol -> dystric cambisol -> spodic cambisol -> podzol. For each soil, two surface hemiorganic horizons (OAh if any, Ah) have been sampled and their major physico-chemical and mineralogical properties have been characterized. A two compartment cropping device (HINSINGER et al., 1992) was used to quantify the absorption of radiocaesium by *Lolium multiflorum* after germination on a K-free nutrient medium. The root mat was placed in contact of a soil(1g)-agar mixture (21 cm^3) for 4 days (5 repetitions). The 12 soil materials were previously saturated by Ca^{2+} and contaminated by $^{137}Cs^+$ (5000 Bq/g soil): the unique K sources are the seedling reserve and the soil "native" K. On separate soil aliquots, $^{137}Cs^+$ extraction was carried out using Na-tetraphenylborate -NaTPB- (SCHULTE and COREY, 1965).

3. Results and Discussion

The fictive "weathering" sequence cambisol -> podzol is characterized by change in humus form *mull -> moder, mor* associated with (i) an increase in the thickness of organic horizons (1->12 cm), organic C in OAh/Ah (1.5->37.1%), soil acidity (pH_{water}: 7.8->3.8), (ii) a decrease in clay content (70->4%) and Total Reserve in Bases (TRB) (268->35 $cmol_c.kg^{-1}$). In mineral horizons, the latter is an estimator of the content in weatherable minerals and therefore of the soil weathering stage. CEC largely varies (8-102 $cmol_c.kg^{-1}$). Illite, vermiculite, HIV and smectite are the major clay minerals in the Cambisols and podzolic soils, respectively. Low Cs rhizospheric mobilization values are measured in the Cambisols (2-12% of the initial $^{137}Cs^+$ contamination). Oppositely, high values are measured in the podzolic soils (9-48%) particularly in the OAh horizons (35-48%). Cs rhizospheric

mobilization is not related to any of the following parameters: CEC, clay and total K content, pH, exchangeable K level. The uptake of $^{137}Cs^+$ depends on the C content, nature of major clay mineral and TRB. The two latest parameters are closely related to each other: the dominance of illite and vermiculite is associated with higher TRB values while that of smectite with lower TRB. Our observations are best fitted by the following linear model:

*Cs rhizospheric mobilization = 0.98*C% - 0.039*TRB + 10.26 (r^2= 0.82)*

In our experimental conditions, plant roots are expected to extract micaceous interlayer K and Cs sorbed on frayed edge sites. This hypothesis is supported by the positive correlation between the relative quantity of $^{137}Cs^+$ extracted by Na-TPB and the $^{137}Cs^+$ rhizospheric mobilization (r^2= 0.84) as illustrated here below.

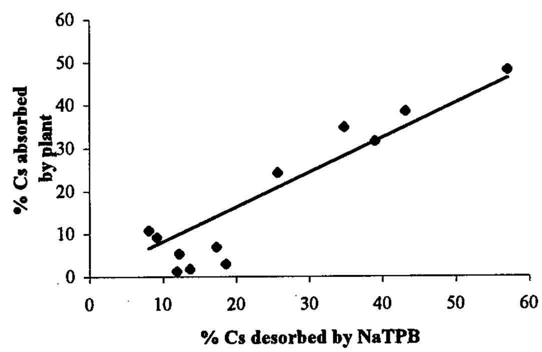

Fig. 1: relative $^{137}Cs^+$ quantity absorbed by plants plotted against relative $^{137}Cs^+$ quantity extracted by NaTPB.

4. Conclusions

The proposed standardized test quantifying the radiocaesium mobilization by plant roots from contaminated soils leads to a categorization of soils with distinct potentials in Cs soil-to-plant transfer. Cs uptake increases with increasing C content and soil weathering stage. The mechanism of Cs rhizospheric mobilization is similar to that of micaceous interlayer K.

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CATION EXCHANGE PROPERTIES OF ROOTS: EXPERIMENTAL AND MODELLING

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1. Introduction

The uptake of major and trace elements by plants is regulated both by active and passive mechanisms at the soil-root interface. The active mechanisms take place in a physico-chemical environment which is depending to a large extent on ion exchange reactions between soil matrix, solution phase and root. Indeed, root can be regarded first as an ion exchanger and therefore acts as a "passive" sink of elements. The aim of this paper is to review briefly the origin, sources of variability and practical implications of root exchange capacity, to present results of exchange equilibria involving aluminium as a model trace element, and to show how a mathematical model can be used to simulate ion fixation on root exchange sites.

2. Origin, measurement, variability and implications of root exchange capacity (REC)

The root exchange sites are essentially located in the apoplasm. Uronic acids from pectin and hemicellulose in cell walls, and proteins to a lower extent, are responsible for the exchange properties of roots. Root exchange capacity can be measured in different ways which can influence the results and consequently the interpretation of data. Basically, as REC mainly originates from carboxylic groups, parameters such as pH, reference cation, ionic strength used in the measurement procedures should be controlled and mentioned. In our laboratory, we have adopted as a reference method a procedure based on Cu adsorption followed by acid extraction (Dufey and Braun, 1986). As the uronic acid content in roots is related to the plant species, REC also first depends on plant species and even on varietes or cultivars of the same species. Monocots have usually smaller REC than dicots. The age of roots or root parts also influences REC. Secondary cell walls very poor in pectin are formed in older root tissues so that the increase of dry weight is not accompanied by a proportional increase of exchange sites. The nutritional status of roots or the presence of toxic elements were also reported to influence REC; this can result from changing the "average age" of the root system. Many authors have attempted to relate REC to plant characteristics such as adaptation to different stresses (saline soils, Al toxicity, low or high soil pH, ...). These studies are not easy to generalize, because they deal almost always with very limited series of plant species or plant varieties. Anyway, it seems that, for example, halophytes have high REC, which can contribute to keep enough Ca on cell walls in Na rich soils. For a given plant species, it seems that varieties with low REC are more tolerant to Al, possibly because Al has a high affinity for exchange sites and impairs physical properties of roots. In a series of 5 banana plant cultivars, we observed that the most Al-sensitive cultivar had REC $\cong$ 30 $\mathrm{cmol_c\ kg^{-1}}$ compared to REC $\cong$ 19-23 $\mathrm{cmol_c\ kg^{-1}}$ for the other 4 cultivars. However contradictory results are reported in literature on this aspect.

3. Fixation of aluminium

Various measurements were performed on roots equilibrated either with solutions coming from acid soils of different types and different pH or from synthetic Al-Ca solutions (Dufey et al., 1991). It was confirmed that the Al quantities fixed by roots are first related to REC. Roots equilibrated with solutions coming from organic soil horizons do not fix much Al even at low

pH. In mineral horizons, Al can dominate the exchange complex of roots so much the more as pH is decreasing. In order to calibrate ion exchange models, Al-Ca exchange isotherms were established for rye-grass, clover, and banana roots, at different pH levels and at different Al-Ca concentrations and fractions.

4. Modelling cation exchange on roots

The model first developed by Sentenac and Grignon (1981) for cell walls was extended to account for different types of exchange sites and different forms of fixed cations. It was generalized to any type of exchanger, i.e. to fixed charge constituents such as 2:1 clays or to variable charge constituents such as organic compounds. It was also coupled with a chemical speciation routine. The model is based on the Donnan theory. This approach is a mid-term between macroscopic cation exchange coefficients expressed as mass-action constants (Gapon, Vanselow), and the detailed description of electrostatic effects in the vicinity of an exchanger with the double diffuse layer theory. The Donnan approach considers mean electrostatic effects and intrinsic dissociation constants between the ions and the exchanger. These constants should be largely independent of experimental conditions. They are related to the nature of the exchangers themselves.

Intrinsic dissociation constants and Donnan volumes were adjusted for R-H, R_2-Ca and R_3-Al complexes where R stands for root exchange site. It was shown that a single set of dissociation constants can be used to simulate with reasonable precision the whole range of experimental conditions covered in this study.

5. Conclusion

As far as trace element uptake is concerned, the effects of physico-chemical exchange reactions between soil solution and root exchange sites should be considered before attempting any interpretation in terms of "active" processes. Plant can accumulate trace elements in roots by passive fixation. However, the effect on total uptake and translocation to shoots is far from elucidated. Clear concepts including soil/rhizosphere chemistry, biophysics and plant physiology are still lacking.

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MODEL CALCULATIONS OF THE DISTRIBUTION DYNAMICS OF ALUMINUM IN THE RHIZOSPHERE OF ACID FOREST SOILS

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1. Introduction

The understanding of tree decline symptoms caused by the chemical conditions in acid soils is still incomplete. Experiments with Al enriched solution cultures, growth chamber experiments and field observations have yielded contradictive results. Soil solution Al concentrations usually do not exceed the identified concentration thresholds of tolerance and Al species of different toxicity have been determined. Hence to evaluate more sophisticated risks of Al rhizotoxicity knowledge about the distribution dynamics of Al species in the rhizosphere is necessary.

2. Modeling approach

The multicomponent model describes the coupled spatio-temporal distribution of all major ions, e.g. H, Al, Mn, Fe, Ca, Mg, K, Na, NH_4, H_2PO_4, NO_3, SO_4, Cl, in the rhizosphere of acid soils. The model assumes that gradients of the chemical concentrations, the diffusion potential gradient, and the transpiration induced water velocity are the driving forces. The model includes the dissociation equilibrium of water, selective cation exchange between soil solution and exchanger, and a cyclic, daily-patterned water flux. The Michaelis-Menten kinetics describe root uptake rates which are used to calculate the H/OH excretion rate by means of the cation-anion uptake imblance. An extended model version considers the formation of Al sulfate complexes and Al hydroxocomplexes.

3. Results

Taking into account previously known parameter values the simulation results show Al accumulations at the root surface due to water fluxes towards the root and decreases during diffusion periods. The Al accumulation is determined by the water flux rate, the root diameter, the back diffusion rate, the soil water content, the NO_3 availability and the extent of buffering by the soil exchanger. Fig. 1 demonstrates the indirect influence of the NO_3 availability on the Al accumulation level. At given bulk soil concentration a decrease of Al ion accumulation at the root surface corresponds to rising NO_3 concentration which is caused by an additional buffering by the soil exchanger. At high NO_3 availability a depletion of protons from soil exchanger sites is due to a neutralization by high rates of root excreted OH ions. The vacant soil exchanger sites are occupied by Al solution ions; consequently, the Al ion solution concentration falls off even at high Al bulk concentration. On the other hand, high concentrations of M_b cations (Ca, Mg, K, Na) and low NO_3 concentration may lead to a

considerable increase of Al soil solution concentration even at low bulk concentrations. This is caused by an increase of H ions in both soil phases due to high H root excretion; hence a slightly higher fraction of Al ions delivered by the water flux remains in soil solution.

4. Discussion and conclusions

It may be concluded that Al soil solution accumulations at the root surface may exeed the concentration threshold tolerable for plant growth. But changing preference of Al ions for rhizosphere soil exchanger sites compared with the bulk soil may change the level of Al accumulation. Even at low Al bulk concentration a low affinity of Al ions for exchanger sites could lead to a toxicological relevant accumulation level and a high affinity may reduce Al solution accumulation considerably, which is intensified by high H exchanger release. Especially at high NO_3 concentration, wet soil conditions and dry soils together with no or reduced water fluxes in the rhizosphere minimize acid stress to roots.

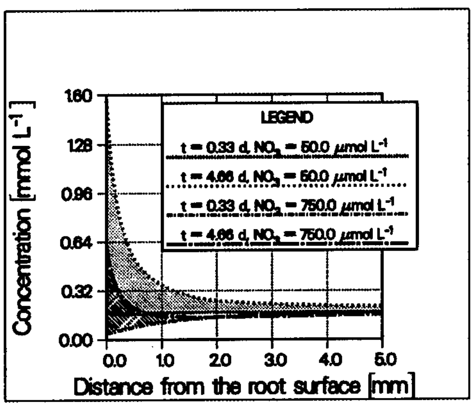

Fig 1.: Ranges of Al solution concentrations at various NO_3 concentrations (boundaries of shaded areas mark concentrations at the end of a diffusion period (0.33d) and at maximum water flux (4.66d))

HEAVY METAL MOBILITY IN SOIL AFFECTED BY SIDEROPHORES AND CITRIC ACID

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1. Introduction

In the rhizosphere, root exudates such as citric acid and microbial chelating agents have a profound influence on metal solubility. For a better understanding of these processes, sorption studies in well-defined model systems were carried out with the microbial siderophore desferrioxamine B (DFOB) as complexing agent.

2. Materials and Methods

Batch sorption studies were carried out with 10ml-samples consisting of 1g/l kaolinite, Na-montmorillonite or goethite, 10 or 80 μM Zn, Cu or Cd (used as nitrate salts) and 300 μM DFOB (used as methyl sulfonate) in 0.1 M $NaClO_4$ or $NaNO_3$. NaOH and $HClO_4$ or HNO_3 were used to adjust the pH. After shaking for two hours on an over-head shaker, the pH was determined. Dissolved metal concentrations were measured by AAS after centrifugation and filtration. Concentration of DFOB was determined using the Cu-Chrome azurol S method by SHENKER et al. (1995). Metal speciation was calculated with published constants from HERNLEM (1996) and ANDEREGG et al., (1963) using the program μQL by GFELLER (1996).

3. Results and Discussion

The presence of DFOB led to a clear decrease in metal sorption on kaolinite. Heavy metals were almost completely kept in solution up to pH 10. In contrast, addition of DFOB enhanced metal sorption on montmorillonite (Fig. 1). A mobilizing effect occured only for Zn and Cd at high pH values, whereas Cu sorption on montmorillonite in presence of DFOB was almost complete over the whole pH range. Ternary complexes including DFOB, metal cations and montmorillonite were probably formed. Sorption studies in absence of metals revealed that free DFOB did not bind to kaolinite but had a high affinity to montmorillonite in the acidic pH range. Above pH 7 DFOB sorption decreased drastically. Speciation calculations showed that interactions between the free ligand and the clay mineral as well as formation of the ternary complexes can be explained by electrostatic interactions. In contrast to kaolinite, montmorillonite carries a permanent negative charge and is therefore attractive for the positively charged DFOB and its metal complexes (see speciation diagram Fig. 2). Adsorption of Cu, Zn and Cd was also enhanced due to DFOB in the system with goethite, however, much less distinct than on montmorillonite (Fig. 1). Since siderophores have a higher affinity to iron(III) than to divalent heavy metal cations, also in this system, ternary complexes could be responsable for the enhanced adsorption. Such ternary complexes would be composed of a heavy metal cation, DFOB and an iron(III) center at the surface of goethite. However, to quantify these interactions, further research is needed to study the effect of DFOB on goethite dissolution. The effect of DFOB on metal sorption decreased for all three minerals in the order Cu > Zn > Cd in accordance with complex stability constants. Further sorption studies will be conducted concerning the influence of citric acid as an example for root exudates.

Batch experiments with contaminated soil samples will show if the postulated mechanisms can be transfered from the model to a real system.

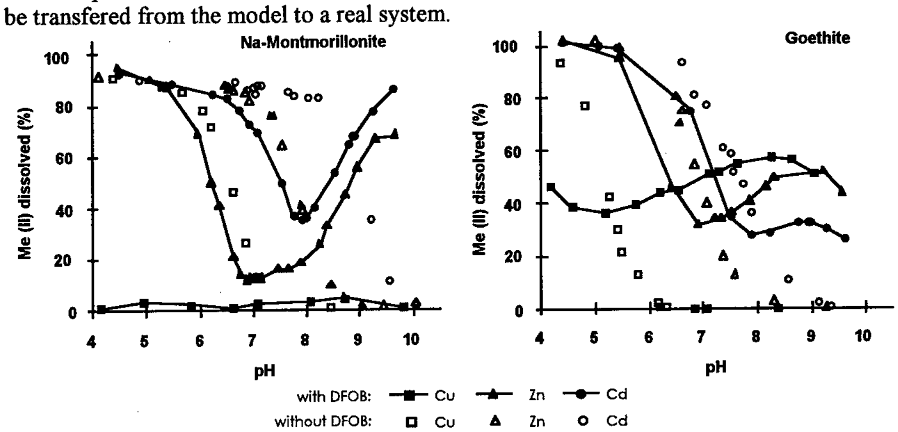

Figure 1: Influence of DFOB on heavy metal adsorption on Na-montmorillonite and goethite.

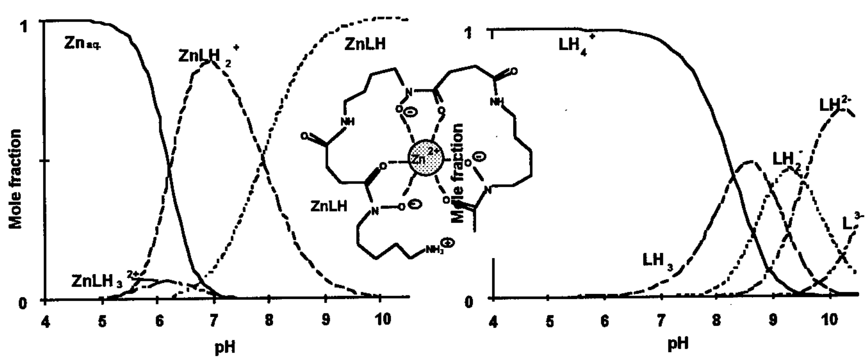

Figure 2: Speciation of DFOB and Zn in aqueous solution. L^{3-} represents the fully deprotonated DFOB. The neutral Zn species shown in the middle is dominant above pH 8.

4. Conclusions

DFOB influenced heavy metal sorption on clay minerals and goethite. Depending on pH, metal speciation and surface charge of the mineral, sorption was either hindered or even enhanced through formation of ternary complexes. The effect decreased in the order Cu > Zn > Cd. Results from sorption studies were found to be in good agreement with speciation calculations.

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THE ROOT/SOIL INTERFACE AT CONDITIONS OF METAL TOXICITY AND NUTRIENT DEFICIENCY: A STUDY EMPHASIZING THE ROLE OF ROOT EXUDATES

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1. Introduction

In order to understand plant tolerance to toxic metal concentrations or metal uptake under nutrient deficiency conditions, the following processes and parameters have to be considered: concentration and chemical form of the metal in the soil matrix and soil solution, the change in root exudation pattern as response to these conditions, the influence of the exudates on metal speciation, reactions of the metal at the root surface, uptake, translocation and storage within the plant. In this study, the complex interactions between roots and soil solution were investigated in hydroponic solutions in detail for two cases, with special emphasis on the role of root exudates.

Investigations on sensitivity to Cu deficiency of plants are mainly on a phenomeno-logical level (Bergmann;1993). From the extensive range of experimental studies dealing with Cu tolerance it was concluded that related phenomena may be considerably more complex than was first envisaged (Baker et al; 1990). A largely missing link in the knowledge of Cu uptake mechanisms is the relation of Cu uptake to speciation and the extent to which plants can regulate Cu uptake by root exudate release. Lupins are known to exude high amounts of citric acid under P deficiency (Marschner, 1995). However, the effect of Cu or Fe stress on the exudation of organic acids by lupins has not been investigated.

It is well known that acid tolerant cultivars of wheat and maize can avoid uptake of Al by exudation of organic acids such as citric and malic acid, that form strong complexes with Al (e.g.Pellet et al, 1995)). For coniferous trees which often are naturally adapted to acid soils, little is known on the mechanisms of Al tolerance. In particular, the role of root exudates has not been investigated so far.

2. Materials and Methods

White lupin (*Lupinus albus* L.) were grown for 7 weeks in treatment solution under semisterile conditions starting from seeds. Treatments were performed with Hoagland solutions which were varied with respect to their Cu (0 = deficiency, 0.5 = reference, 10, 50, 200 µmol/L), Fe (0 = deficiency, 100 µmol/L = reference) and P (0 = deficiency, 1 mmol/L = reference) concentration.

Ectomycorrhizal Norway spruce (*Picea abies* (L.) Karst.) plants grown for 3 years in a substrate composed of garden peat and wood chips were treated for 1 week with 500 µM

$CaCl_2$ or $AlCl_3$ keeping a constant pH of 4. The treatment solution was replaced with fresh solution after 2 and 4 days.

Treatment "solutions" at different times during the treatment were separated into particulate, high molecular and low molecular dissolved fractions. The soluble fractions were analyzed for dissolved organic carbon, organic acids and total phenolics. At the end of the treatments, the distribution of Cu or Al between the different "solution" fractions and the plant compartments was determined.

3. Results and Discussion

After treatment of white lupin with elevated concentrations of Cu higher amounts of phenolics were found in the low molecular dissolved as well as in the particulate fraction (polyphenols) of the treatment "solution" when compared with the reference. Copper deficiency treatment resulted in a higher release of dissolved organic carbon which is currently investigated in more detail. Neither treatment significantly influenced the release of small organic acids, the most important being glycolic acid. Plants exposed to Fe deficiency acidified the nutrient solution compared with reference plants. In addition, they released phenolic compounds into the soluble fraction, but only little particulate material was found. Elevated Cu concentrations in the medium were reflected by higher Cu contents in the plants. Cu was mainly accumulated in the roots, but there was also a transfer to the shoots. Cu accumulation in the roots was further observed under Fe deficiency conditions.

Treating spruce with Al or Ca, no difference in the release of total dissolved organic carbon and of total phenolics was observed. However, fluorescence spectroscopy provided evidence for complexation of Al by phenolics. In addition, strong evidence was found for an increased release of oxalic acid in the presence of Al. However, the oxalate concentrations were much too low as to play a significant role in Al detoxification. During the first treatment cycle, most of the added Al was adsorbed to the root surface in a rapid exchange with nutrient cations. At the end of the 7 days treatment, large amounts of Al were found in the outer parts of the root in both exchangeable and acid extractable form. In addition, at the end of the second and third treatment cycle, a significant part of Al was found in the high molecular weight fraction. Based on the fluorescence spectroscopy results, the high molecular Al was attributed to aggregates between Al and phenolics.

4. Conclusions

The results from the studies on white lupin and ectomycorrhizal Norway spruce suggest a role of released phenolics in the tolerance of both plants to toxic metal concentrations. While lupin seems to actively increase its exudation of phenolics in the presence of high Cu concentrations, Norway spruce probably is protected from toxic Al concentrations passively by formation of high molecular weight aggregates between Al and phenolics and by adsorption of Al to phenolics precipitated at the root surface.

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REMOVAL OF CADMIUM BY HYPHAE OF ARBUSCULAR MYCORRHIZAL FUNGI

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1. Introduction

Arbuscular mycorrhizae (AM) is a widespread symbiosis between plants and fungi, where the fungus grows inside the roots and into the soil contributing to the transport of nutrients to the plant. External hyphae may also transport non essential elements like cadmium. Mycorrhiza lead to a higher retention of adsorbed Cd in the roots, reducing concentration in shoots (Joner and Leyval, 1996). However, the mechanism behind heavy metal fixation in mycorrhizal crop plants is unknown (Leyval et al. 1997). For ectomycorrhizal fungi, the retention of heavy metals in fungal structures within the roots has been documented (Turnau et al, 1996). The objective of the present work was to study the capacity of extraradical hyphae for adsorption and absorption of heavy metals and competing cations. It was necessary first to set up an experimental device where large quantities of extraradical mycorrhizal hyphae would be produced and could be easily collected.

2. Materials and Methods

Pure excised hyphae in sufficient quantities were produced in an experimental device after Johansen et al. (1996). Clover was grown and inoculated with mycorrhizal spores of *Glomus mosseae* in the inside part of two-compartment pots and kept in a growth chamber. After 6 to 8 weeks, extraradical hyphae could be extracted from the outside compartment filled with sand, by rinsing the sand and collecting the hyphae on a 60 micron sieve. Excised hyphae were used to quantify absorption/adsorption of cadmium, by putting the hyphae in tubes containing increasing Cd concentrations and measuring Cd remaining in solution. The influence of buffering the medium, and of the simultaneous exposure to Ca and Zn was investigated.

3. Results and Discussion

The compartment device showed to be successfull with different AM fungi and after 6 to 8 weeks, approximately 100 mg fresh weight mycelium could be collected every week from each pot.

Cd in solution in the presence of excised hyphae rapidly decreased and was stable after one hour. The immobilization of Cd by hyphae differed with different isolates of *Glomus mosseae* and decreased when the medium was buffered (Fig.1). The presence of Ca and Zn drastically decreased the immobilization of Cd by hyphae suggesting a competition for binding sites, as observed for filamentous bacteria (Shuttleworth and Unz, 1993). The results confirm that AM fungi can contribute to alleviate metal toxicity to plants by immobilizing metals in fungal structures. Genetic engineering of plants to improve their tolerance to metals may play an

important role in the future (Leyval et al, 1997). The management of micro-organisms closely associated to roots such as mycorrhizal fungi should also be considered.

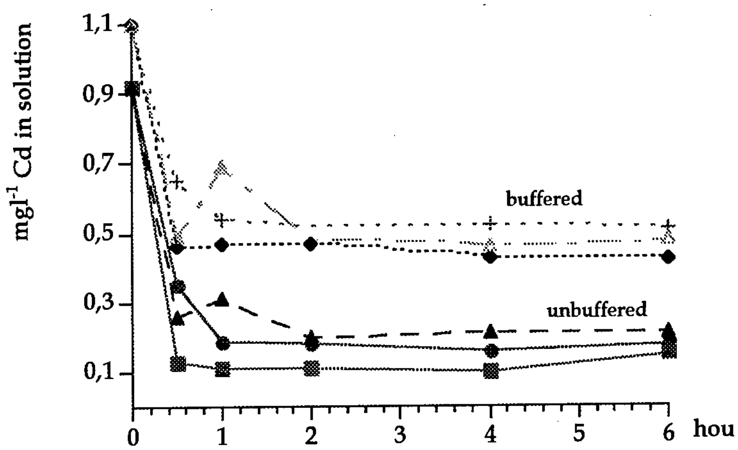

Fig. 1: Adsorption of cadmium on mycorrhizal hyphae of *Glomus mosseae* (Gm)

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OCCURRENCE OF MYCORRHIZAE AND ROOT NODULES IN PLANTS GROWING ON TANNERY EFFLUENT POLLUTED SOILS

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1. Introduction

Heavy metal contaminated land is increasingly becoming an important environmental, health, economic and planning issue in Pakistan (Hussain *et al.* 1996). The rapid increase in population together with the unplanned disposal of industrial effluent have increased the threat of soil pollution in Pakistan. There is a need to gather some basic data regarding the physical, chemical and biological parameters of heavy metal polluted soils in Pakistan before applying suitable rehabilitation strategies.

Studies by the Pakistan EPA (EPA, Lahore, 1990) have shown value for chromium in the tannery effluent many folded compared with the safe limits of the national standard. The damage to the environment caused by hazardous tannery effluent is becoming an acute problem in Pakistan. The main objectives of this research are to report the presence of trees growing on the chromium contaminated site near a tannery in the Punjab and the association of their roots with symbiotic nitrogen fixing bacteria and mycorrhizal fungi and to discuss their potential in phytoremediation.

2. Materials and Methods

Roots and the rhizosphere soil samples of trees, namely *Populus euroamericana*, *Acacia arabica* and *Delbergia sissoo*, growing in the contaminated soils near the tannery and those growing in the nearby non-contaminated reference site, were collected. The pigmented root pieces were cleared, and stained to determine the mycorrhizal infections (Brundrett *et al.* 1966). Presence or absence of functional nitrogen fixing nodules on the root pieces were also recorded. Rhizosphere soil samples were wet sieved and decanted to recover arbuscular mycorrhizal fungal propagules (Brundrett *et al.* 1996). The mycorrhizosphere soil samples from the contaminated as well as the reference sites were analyzed for total Cr contents (extracted by concentrated HNO_3 and HCl acids, with small aliquots of 30% hydrogen peroxide) and plant available Cr (0.005M EDTA extraction) by using flame atomic absorption spectrometry (Carter, 1993).

3. Results and Discussion

Mycorrhizosphere soil samples from the reference site contained significantly lower total Cr. content (80 ± 18 mg kg $^{-1}$) as compared to those from the effluent polluted soils (200 ± 40 mg kg $^{-1}$). Values for the DTPA extractable Cr in the tannery effluent polluted soils (630 mg kg $^{-1}$)and in the reference site (180 ± 26 mg kg $^{-1}$) were also significantly different ($P < 0.05$). Roots of all the species from both the contaminated and the reference sites examined were found to be mycorrhizal. Ectotrophic mycorrhizal associations were also observed in a

few roots of *Populus euroamericana*. A 5-7 μ thick mantle covered the fine rootlets of popular. Root cortices of all the trees examined contained vesicles and a few arbuscules in the innermost cell layers, i.e. they possessed arbuscular mycorrhizae (AM). The infection ranged from 51-71%, 34-60% and 70-80% in the roots of *D. sissoo, P. euroamericana,* and *A. arabica,* respectively. The roots of the trees growing on the non-contaminated reference site were also mycorrhizal (>80%). Roots of all the species were heavy pigmented and required bleaching. This may have reduced the subsequent staining of the delicate hyphal branches of arbuscules, explaining the low frequencies of arbuscular infections observed. These observations are consistent with those of other researchers (Khan *et al.* 1998). The mycorrhizosphere samples from all the trees were found to harbour AM fungal spores belonging to *Gigaspora* strains. The AM fungal flora in the rhizosphere of the trees growing on the reference site consisted of mainly *Glomus* strains. The roots of *D. sissoo* and *A. arabica* trees, growing on both the contaminated as well as the reference site, had dual symbiotic associations, i.e. AM infection as well as typical leguminous root nodules containing nitrogen fixing *Rhizobium* bacteria. The dual infection would encourage mineral nutrition, including heavy metals, and increase growth (Bethlenfalvay,1992).

4. Conclusions

The occurrence of symbiotic associations in the trees growing on the polluted areas studied suggests a selective advantage for these plants as pioneer species on heavy metal contaminated soils and responsible for their successful colonization and phytoremediation of such habitats.

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METAL UPTAKE IN ECTOMYCORRHIZAL BIOMASS *IN VITRO*

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1. Introduction

Ectomycorrhizal biomass from contaminated forest surface soils show a large ability to accumulate metals such as Zn, Cu and Cd (Berthelsen et al., 1995). How and to what extent metal uptake affects growth of mycorrhizal biomass has been subject to several investigations, although mainly at high exposure levels with respect to metals (e.g. Colpaert and van Assche, 1992). The purpose of the present study was to investigate uptake of Zn, Cu, Pb and Cd into ectomycorrhizal biomass *in vitro* at moderate exposure levels as well as studies of growth effects on fungal biomass due to metal exposure.

2. Materials and Methods

Homogenates from liquid cultures of 3 mycorrhizal fungi, *Cenococcum graniforme* (Cg), *Suillus variegatus* (Sv) and *Piloderma croceum* (Pc), were inoculated in separate wells on Microtiter plates using standard MMN medium at pH 4.5-4.7, and exposed to 3 levels of a mixture of Zn, Pb, Cu and Cd (Table 1) in addition to a control (MMN medium and de-ionized water). Each combination of fungal type and treatment was replicated 8 times.

Table 1: Metal concentrations in the different exposure solutions

TREATMENT	METAL CONCENTRATIONS (µG/L) ZN	CU	PB	CD
1	400	400	200	40
2	800	800	400	80
3	1600	1600	800	160

Biomass growth during the experiment was measured every day as net Optical Density (OD) growth for each well. Fungal biomass was harvested at 3 times after metal exposure for metal analyses: Sv (13, 15 and 22 days), Cg and Pc (30, 34 and 44 days). Maximum net growth appeared 13 days after metal addition with respect to Sv and 30 days after exposure with respect to Cg and Pc.

3. Results and Discussion

At present no metal analyses have been performed. The 3 species showed large differences concerning growth effects due to metal exposure as evident from OD measurements (Table 2).

Table 2: Net OD growth (arithmetic means) for each fungus and different metal treatments at max. biomass growth for each species. Errors are shown as standard errors (n = 48).

Fungus	Control	Net OD growth for different metal treatments 1	2	3
Sv	1.246±0.013	1.129±0.009	1.005±0.008	0.980±0.007
Cg	1.869±0.011	2.060±0.011	2.142±0.014	2.183±0.013
Pc	**1.235±0.004**	**1.229±0.003**	**1.231±0.004**	**1.248±0.005**

The growth of *Piloderma croceum* was not notably influenced by metal additions. The growth of *Suillus variegatus* decreased with increasing metal exposure (Table 2). On the contrary, the growth of *Cenococcum graniforme* seemed to be stimulated by metal addition, showing increased growth with increasing metal exposure. Metal analyses may reveal whether differences concerning growth effects on the 3 fungi are related to amounts of metals taken up into fungal tissue. The results from Table 2 indicate that Cg and Pc are more metal tolerant than are Sv, which may be related to biosynthesis of metal binding proteins, as evident for other mycorrhizal fungi (Howe et al., 1997).

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INTENSIFICATION OF NODULATION AND NITROGEN FIXING ACTIVITY PRECEDING THE "LOSS OF FUNCTION" BY THE LONG-TERM APPLICATION OF SOME TOXIC METAL RATES

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1. Introduction

Due to the long-term sewage sludge application the accumulation of heavy metal salts can effect the sensitivity of some N_2-fixing microorganisms, such as blue-green algae and *Rhizobium* bacteria (BROOKES et *al.* 1986 and McGRATH et *al.* 1988). After about 15 years of application the toxicity resulted in a decreased nitrogen-fixing ability of clover and a reduced effectivity and diversity of rhizobia (GILLER et *al.* 1989). Using the sewage sludge, however a mixture of various toxic metals are also given, where the separate effects of metals (or especially of the different rates) are not really known.

2. Materials and Methods

Long-term field experiment was set up in 1991 on a calcareous loamy chernozem soil, by a single mass application of 13 microelement salts (Al, As, Ba, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Se, Sr and Zn) in 4 rates (0/30, 90, 270 and 810 kg ha^{-1}).

The symbiotic performance (nodulation, acetylene-reduction activity-ARA) developed by the indigenous *Rhizobium* bacteria was tested in 1994 on the green pea (*Pisum sativum* L.) besides some other characteristics (shoot, root and seed biomass, plant height, macro- and micro-element content of the host plant and the soilwere determined. More details in KÁDÁR, 1998 (in this issue).

3. Results and Discussion

Table 1. *Total number of root nodules on pea (Pisum sativum) at the various toxic elements in a long-term field experiment (Nagyhörcsök, Hungary).*

Toxic elements	Originally applied metal rates (kg ha^{-1}) Nodule number (piece/100 plants)					
	$0/30^+$	90	270	810	$LSD_{5\%}$	mean
Al	16.1	13.5	13.2	**39.7**		21.1
As^+	27.9	**53.1**	19.2	16.0		29.0
Ba	18.7	20.9	**37.5**	18.5		23,8
Cd^+	20.7	14.7	23.9	**29.2**		22.2
Cr	20.3	25.2	**53.4**	22.6		30.3
Cu	12.7	14.5	16.4	13.6		14.3
Hg^+	19.0	24.2	**53.1.**	29.7	20.2*	30.3
Mo	34.6	28.7	41.2	**41.5**		39.1
Ni	17.1	31.4	**80.4**	17.4		36.5
Pb	20.5	29.7	27.0	31.5		27.1
Se^+	**36.0**	8.7	5.1	6.5		14.1
Sr	19.7	**48.2**	15.2	15.5		24.5
Zn	26.7	17.5	16.4	**61.2**		33.2
mean	20.0	23.8	30.9	34.3		25.0
$LSD_{5\%}$	24.3*				7.2	14.4*

Among the 13 toxic elements a significant increase of nodulation was found depending on the toxicity of the various applied rates to the soil. According to this finding the enhanced nodule number has varied between 36 and 80, comparing to the 20 as an average number on the control (100) plants. At 30 kg ha^{-1} rate was the highest nodulation found for selenium, which proved to be the most toxic elements at the further concentrations. In case of Al and Zn, however rate 810 kg ha^{-1} resulted the highest nodule number.

Acetylene-reduction activity (ARA) proved to be especially high also at a low level (90 kg ha^{-1}) of Se application (Table 2). Other elements, such as As, Cd and Cr at 90 –810 kg ha^{-1} rates showed also a slight increase as a tendency of intensification, although it was not significant.

Other parameters, such as the plant height and biomass has showed the same tendency of increasing than the nodulation to a certain point depending on the toxicity of various metal-loadings (data not shown).

Table 2. *Increased acetylene reduction activity (ARA) of pea roots at some field applied metal rates (ng ethylene/pot/2hours)*

Metals	originally applied metal rates kg/ha				
	0/30⁺	**90**	**270**	**810**	**mean**
As⁺	87.9	**223.3**	115.9	143.7	**182.7**
Cd	64.2	86.8	91.8	**116.2**	89.9
Cr	103.8	76.9	72.9	**133.6**	94.5
Se⁺	161.6	**1831.1**	77.3	80.0	**535.1**
$SzD_{5\%}$	446.5*				507.4*

4. Conclusions

The enhanced nodulation characteristics and also the plant-size-biomass parameters are increasing to the optimal level of the applied microelements in a certain soil system. In this processes not only the application rates, but also the effecting periods are also important. Testing the nitrogen-fixing parameters it must be possible to realise the decreasing tendency preceding the total loss of beneficial nitrogen-fixing functions. *Rhizobium* microsymbionts are sensitive enough for developing a reliable system for the identification of heavy metal pollution.

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BEHAVIOUR OF MICRONUTRIENTS IN THE RHIZOSPHERE

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1. Introduction

Behaviour of micronutrient around plant root was investigated using rhizobox system. The rhizobox system (Youssef and Chino 1989) consists of several compartments made of plastic frame with a nylon cloth (200mm/200mm/1mm) having a radius pore <25um stretched across it. The central compartment (CC) consists of a plastic frame (200 mm/200 mm/2 mm) with the same arrangement where plants are grown. In this rhizobox system, CC is designated as 0 mm compartment and each of the six compartments on the left and right sides of the CC are designated as 0-1 mm, 1-2 mm, 2-3 mm, 3-4 mm, 4-5 mm and >5 mm compartments (bulk soil compartments). The characteristic of this rhizobox tool is that the nylon cloth restricts root growth successfully within the CC compartment and thus ensures individual sampling of soil fractions differently affected by the roots at defined distances of the respective compartments of the rhizobox.

2. Materials and Methods

Three experiments were conducted to know how chemical forms of the nutrients and plant species affect the behaviour of micronutrients in the rhizosphere..

In the first experiments, the behaviour of cations in the wheat rhiosphere was investigated. Wheat seedligs grew in the central compartment. After two months of cultivation, all the compartments were separated from the rhizobox and the soil from each compartment was dried and analyzed for the element content. The plants were also harvested and analyzed for the content of the elements.

In the second experiment, the effects of chemical form of Zn and Mn added to the soil on the behaviour of micronutrient elements around the wheat roots were investigated using the rhizobox system. Mn and Zn were added either as water-soluble form ($MnCl2$ and $ZnSO4$) or citrate-soluble form (fritted Mn and Zn). Wheat seedlings were grown in C.C. of the rhizobox. After 40 days of cultivation, all the compartments were separated from the rhizobox for the analysis of the element composition.

In the third experiment, behaviour of micronutrients in the rhizosphere of different plant species(soybean abd barley) and cultivars (Fe resistant and sensitive cultivars of soybean) was investigated. Soybean and barley seedlings grew in the C.C. compartment. After two months of cultivation, the soil from each compartment of the rhizobox was analyzed for the element conposition.

3. Results and Discussion

The results obtained were:

1) The pH values across the rhizosphere of wheat roots decreased by about 2.5 units. The significant decrease in pH was observed about 2 mm from the central compartment.
2) Total Cu, Zn, Mn, Fe, Mg contents in each compartment showed little or no difference, but total Ca content increased slightly in the central compartment.
3) Most of soluble cations (Mn, Fe, Ca, Mg, and Na) increased near the root, while the content of soluble K+ and NH4+ decreased.

The results obtained in experiment 2 were as follows:

1) Similar changes in pH in rhizosphere due to plant cultivation were observed as in the Experiment1. The chemical forms of Mn and Zn added to the soil did not influence the pH of the soil so much.
2) Soluble Zn decreased while other soluble heavy metals (Mn, Fe and Cu) increased near the root in the treatment of zinc sulfate or of mixture of zinc sulfate with mangnese chloride. On the other hand, the application of citrate-soluble Zn and Mn increased the content of all soluble elements (Zn, Fe, Cu and Mn) near the root.
3) Distribution of elements in the wheat plant was studied. The concentration of Zn of roots had intimate relations to that of DTPA-soluble Zn of the soil of C.C. of each treatment. When cirate-soluble Zn was added to the soil, the ratios of Zn concentration of the top to that of the root were generally higher than those when water-soluble Zn was added. For other elements, no significant influences due to the difference of chemical form of Zn and Mn were observed.

The results obtained in experimet 3 were:

1) Similar changes in pH were also observed in all the treatments as in the former two experiments. The significant decrease in pH of the soybean roots was observed 3-4 mm from the central compartment. But, the significant decrease in pH of the barley was observed in the 4-5 mm compartment.
2) Most of soluble cations (Mn, Fe, Ca, Mg and Na) increased near the root, while the content of soluble K and NH_4 decreased. The solubilization of Fe by the bulk soil and by the rhizospheric soil was compared. The capacity of rhizospheric soil of Bride B216 soybean to solubilize Fe and Mn in the soil was higher than that of Hawkeye soybean.

4. Conclusions

The rhizobox system allows for assessment of root-affected rhizosphere phenomena such as pH changes , microbial activities and nutrient distribution including micronutrients between the rhizosphere and bulk soils. Dependent on the experimental conditions, micronutrients show distinct pattern of distribution and fractionation in the rhizosphere of wheat.

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EFFECT OF IRRIGATION USING SEWAGE WATER ON THE DISTRIBUTION OF SOME HEAVY METALS IN BULK AND RHIZOSPHERE SOILS AND DIFFERNT PLANT SPECIES: CABBAGE PLANTS (BRASSICA OLERACEA L.); ORANGE TREES (CITRUS SINENSIS L.)

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1. Introduction

The use of sewage water and sludge in agriculture have been old practices that are developed world wide. Such practice have resulted in an accumulation of heavy metals in soils (McBride, 1995). However, the concentration of heavy metals in a soil vary in the different regions (rhizosphere vs. bulk soil). Plant species can differ in their metal uptake and in their effect on rhizosphere and bulk soil heavy metals concentrations (McGrath et.al., 1997). The dynamic changes in the rhizosphere depends on plant species, rate of nutrients uptake, transpiration rate, cation/anion uptake ratio and root cation exchange capacity (Marschner, 1986).The objectives of the present study are to evaluate: the effect of irrigation with sewage water on rhizosphere and bulk soils chemical characteristics; and the distribution of heavy metals in the rhizosphere and bulk soils and plant organs.

2. Materials and Methods

Plant and Soil sampling and analysis:

Cabbage plants and samples from orange trees were collected from El-Gabal ElAsfar farm, which have been irrigated with raw sewage water, during the winter season of 1998 at three locations, represents the following irrigation periods (10, 40, and 80 years). Cabbage plants were separated into leaves, stems, and roots and orange trees organs included leaves, roots, and fruits (peel and pulp). Plant organs were washed with tap water, rinsed with deionized water, oven dried at 60 °C then ground using micromill. Heavy metals content were measured after digestion with HNO_3-H_2SO_4-$HClO_4$ acids mixture (Jackson, 1967). Surface soil samples (0-30 cm) were collected from the same locations where plants were collected. As for the rhizosphere soil, it was collected by removing the soil layer of ~ 2-5 mm from the surface of the root axis. Then the soils were air dried, crushed to pass 2 mm sieve and kept for analysis. Soil general characteristics were determined using standard methods outlined by Jackson (1967) methods of analysis. Total heavy metals contents were determined after digestion with H_2SO_4 + $HClO_4$ acid mixture (Jackson, 1967). Available heavy metals contents were extracted using 0.005 M DTPA solution (Lindsay and Norvell, 1978) and 0.5 N HNO_3. Soil and plant heavy metals were measured using the Atomic Absorption Spectrophotometer (GBC 932).

3. Results and Discussion

Soil General Characteristics:

The continous irrigation of soil cultivated with cabbage plants and orange trees increased OM, clay, and EC contents and decreased $CaCO_3$ and pH in both rhizosphere and bulk soils, comparing with the check soil. The rhizosphere contain lower pH values than the bulk soil. The rhizosphere soil of orange trees exhibited lower pH values than the rhizosphere soil of cabbage plants. This difference between the rhizospheres pH could resulted from more acidic exudates being released from orange trees roots.

Heavy Metals in the Soil:

The total and available heavy metals content show continous increase as the irrigation periods inceased in soil cultivated with cabbage plants and orange trees comparing with the check soil. These contents of the rhizosphere soil cultivated with orange trees are higher than the rhizosphere soil cultivated with cabbage plants. Soil cultivated with cabbage plants are exposed to continous turning of the soil surface during tillage which could resulted in migration of heavy metals from the surface to the subsurface soil. Under the same irrigation period and the same plant species there are tremendous increase in the DTPA–extractable heavy metals of the rhizosphere soil comparing with the bulk soil. Such increase could

be due to the different chemistry of the rhizosphere soil from the bulk soil, having lower pH values which resulted in higher solubility of heavy metals. Youssef and Chino (1989) stated that sludge treatment increased the solubility of Zn, Fe, Mn, in the rhizosphere of sandy soil. A highly significant correlation were found between the DTPA-extractable and both HNO_3 –extractable and total heavy metals contents.

Table (1): DTPA-extractable heavy metals (μg/g) in the rhizosphere and bulk soils irrigated with sewage water for different periods

Metals	Location	Check soil	Soil cultivated with cabbage Irrigation periods (years)			Soil cultivated with orange trees Irrigation periods (years)		
			10	40	80	10	40	80
Fe	Rhizosphere	-	15.20	31.80	58.40	23.3	37.7	65.1
	Bulk	2.50	10.30	17.90	37.40	12.8	21.6	54.7
Mn	Rhizosphere	-	12.50	24.50	44.30	17.5	34.0	57.5
	Bulk	1.85	10.80	17.80	27.30	11.3	23.0	31.3
Cu	Rhizosphere	-	4.00	7.70	16.50	9.60	13.4	26.3
	Bulk	0.12	2.50	4.30	10.30	3.60	7.90	17.3
Zn	Rhizosphere	-	15.70	35.80	70.40	27.5	52.5	90.6
	Bulk	0.45	13.20	20.10	54.30	17.4	45.5	70.6
Cd	Rhizosphere	-	0.07	0.16	0.21	0.09	0.19	0.25
	Bulk	0.02	0.04	0.10	0.17	0.07	0.12	0.20
Co	Rhizosphere	-	1.40	1.70	2.10	1.65	2.10	2.70
	Bulk	0.10	1.10	1.50	1.90	1.35	1.80	2.20
Pb	Rhizosphere	-	2.40	3.55	7.63	3.39	8.50	14.3
	Bulk	0.20	1.97	2.73	7.34	2.62	6.87	10.5

Plant heavy metals contents:

The accumulation of heavy metals in cabbage plants and orange trees have increased as the irrigation periods increased.The descending order in plant organ's heavy metals concentrations are as follows: roots> leaves> stems for cabbage plants and roots> leaves> peels> pulps for orange trees. This data indicate that the translocation processes of heavy metals into orange fruits was lower than in the other tree organs (e.g. leaves). Cabbage roots contain lower heavy metals than roots of orange trees, however, cabbage leaves exhibited higher heavy metals content than orange trees leaves. This could be due to orange trees leaves are expected to maintain lower transpiration rate than cabbage leaves ,since the upper side of citrus leaves are without stomata and transpiration are largely cuticular, Walter et.al., (1968).

4. Conclusions

The prolonged effect of using sewage water in irrigation was reflected in an increase of soil OM, and total and available heavy metals and a decrease in soil pH. These changes were more pronounced in the rhizosphere soil than the bulk soil. The rhizosphere region of soil cultivated with orange trees maintained lower pH values and higher heavy metals concentrations than the same region of soil cultivated with cabbage plants. Heavy metals concentrated mainly in the roots, however the concentrations are higher in orange trees roots than cabbage roots. Whereas their accumulation are higher in cabbage leaves than orange trees leaves. We suggest that the use of sewage sludge and/or sewage water should be avoided in growing leafy vegetables.

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UPTAKE OF ZN AND CU BY MYCORRHIZAL *LEUCAENA LEUCOCEPHALA* FROM HEAVY METAL POLLUTED SOIL UNDER GREENHOUSE AND FIELD CONDITIONS OF THE NAHDA REGION OF EGYPT.

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1. Introduction

The population in developing countries is growing rapidly with a corresponding increase of heavy metal pollution of surface waters and soils. An example of recent heavy metal pollution due to the application of canal sediments for soil amelioration was demonstrated in the Nahda area, north west of the Nile delta of Egypt. In addition to this development, nitrogen fixing trees such as *Leucaena leucocephala* are increasingly used as green manure, especially on low fertility soils where mycorrhizal associations play a significant role.

The ability of vesicular arbuscular mycorrhizas (VAM) to enhance plant uptake of P and N has been demonstrated. Contrary to major nutrients, the results about the effect of VAM on the uptake of trace elements by plants are conflicting. Some reports indicate higher concentrations whereas others found a reduced trace element concentration in mycorrhizal plants. This suggests that some important factors are often overlooked. In our opinion, little attention has been so far devoted to the influence of VAM on root development and soil rooting density, which may affect the uptake of trace elements in pot experiments and in the field differently. Against this background the effect of VAM inoculation on the uptake of Zn and Cu by *Leucaena leucocephala* under field conditions was compared with pot experiments.

2. Materials and Methods

Some characteristics of the Nahda II alluvial sandy soil are summarized in Table 1. A *Leucaena* stand was established in the field from February to August 1995. During the same period, pot experiments were conducted under greenhouse conditions using soil material from topsoil as substrate. In both field and pot experiments VAM inoculated (two *Glomus species*) and noninoculated treatments were compared in eight replicates. By the end of August 1995, the shoot were cut about 25 cm above the soil surface for analysis. Field soils were sampled by auger while whole roots were collected from the pots for root investigations. Further experimental details were described by Helal 1997.

3. Results and Discussion

Mycorrhizal inoculation enhanced plant growth, root development and P uptake both in pot and field experiments significantly (Table 2). Increased rooting density of the field soil was especially evident in subsoil. The mycorrhizal inoculation affected Zn and Cu concentrations in Leucaena shoots in pot and field experiments differently, however. In pot grown plants, both Zn and Cu concentrations were significantly higher in the VAM treatment compared to non-inoculated plants. Contrary to this, the Zn concentration was hardly affected while the Cu concentration was reduced through VAM inoculation in the field. The reasons for this differential effect of VAM inoculation is not clear but may be related to modified root distribution in field soil.

Table 1. Soil characteristics at Nahda II

	Clay	pH	Organic -C,	Available - P	Total - Zn	Total - Cu
	$g\ kg^{-1}$		$g\cdot kg^{-1}$	$mg\cdot kg^{-1}$	$mg\cdot kg^{-1}$	$mg\cdot kg^{-1}$
Topsoil (0-30 cm)	60	7.9	7.4	52	24	15
Subsoil (30-60 cm)	110	7.5	4.3	21	11	8

Table 2. Root development, shoot biomass and P, Zn and Cu concentration in the shoots of *Leucaena leucochephala* as affected by mycorrhizal inoculation in pot and field experiments.

	a) Pot		b) Field	
	(-) VAM	(+) VAM	(-) VAM	(+) VAM
Shoot dry weight, a) $g\cdot pot^{-1}$, b) $g\cdot m^{-2}$	72	96*	310	425*
Root length density, $cm\cdot cm^{-3}$, Topsoil	6.6	9.3*	3.5	3.7
Subsoil			1.3	2.9
% Infected roots	12	47*	17	38*
% P, Shoot	0.17	0.28*	0.19	0.31*
Shoot Zn, $mg\cdot kg^{-1}$	21	34*	18	19
Shoot Cu, $mg\cdot kg^{-1}$	13	18*	12	7*

*) Significantly different from the (-) VAM treatment at P 0.05 level.

4. Conclusion

This work demonstrates the complexity of mycorrhizal-heavy metal interactions and suggests that enhancing subsoil rooting by mycorrhizal plants may provide a possibility for partial avoidance of metal enriched topsoil.

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RHIZOSPHERE – CONTAMINANT INTERACTION AND ITS ROLE IN PHYTOREMEDIATION: A REVIEW

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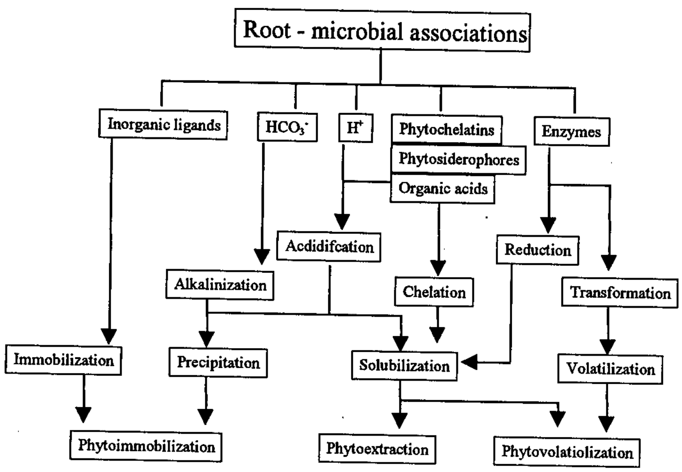
1. Introduction

A focal point of soil plant interactions is the microenvironment surrounding the plant roots, the rhizosphere. This microecosystem is characterized by physical, chemical, and biological conditions that differ from the bulk soil. These are created by the plant roots and their microbial associations. These rhizosphere-related biogeochemical processes are considerably influenced by edaphic and climatic conditions. The edaphic influence is in turn modified by soil physical, mineralogical, chemical, and biological features. Only most recently the role of rhizosphere processes in phytoremediation of heavy metal polluted soils has been addressed in experiments.

2. State of the art

Based on a literature review of metal interactions with plant - microbial associations in the rhizosphere we derive a flowchart presenting the major processes that may be beneficial for the rhizosphere-based phytoremediation technologies:



Phytoimmobilization of metal cations will be primarily supported by alkalinization of the rhizosphere soil due to release of HCO_3^-. However, oxyanions of metals (e.g. Mo, Cr) and

metalloids (e.g. As, Se) will be immobilized by release of protons into the rhizosphere. For Pb, the formation of a Pb phosphate mineral has been identified to cause precipiation and thus immobilization. Accordingly, the accumulation of inorganic ligands in the rhizosphere may be effective in phytoimmobilization.

Phytoextraction will benefit from rhizosphere processes primarily through plant - microbe induced solubilization prior to uptake by the plant. Excretion of protons and exudation of organic compounds carrying acidic functional groups, such as organic acids, can decrease the pH and thus solubilize metal cations. Metal cations are also solubilized through the formation of metal-organic chelates with phytosiderophores and organic acids released by plant roots or microbes. Reductive enzymes may play a role in the solubilization of As, Cu and Mn, while Cr is immobilized. Metalloids such as Se and As can be solubilized upon alkalinization due to excretion of carboxylic acid by roots or microbes.

McGrath et al. (1997) studied the rhizosphere of *Thlaspi caerulescens* (a Zn and Cd hyperaccumulator) and *Thalspi ochroleucum* (a tolerant plant). *T. caerulescens* decreased the concentration of mobile Zn more than *T. ochroleucum*. The decrease in the mobile fraction was responsible only for <10% of the total Zn uptake by the hyperaccumulator plant. Knight et al. (1997) found similar results and postulated that the ability of *T. caerulescens* to accumulate Zn was probably due to its capacity of mobilize Zn or to the large capacity of the soil to buffer the soil solution Zn. The ability of hyperaccumulator plants to take up large amounts of metals can be also related to differences in the specificity and number of metal transporters in the roots. Lasat et al. (1996) found a higher density of Zn transporters per unit of membrane area in *Thlaspi caerulescens* roots compared to *T. arvense*.

Several studies have shown the potential of adding synthetic chelates to Pb and Cd contaminated sites to increase metal accumulation in plants (Blaylock et al. 1997; Huang et al. 1997). The efficiency of added chelating agents supports our view that the release of natural chelating agents in the rhizosphere of phytoremediation crops may be a focal point in the development of phytoextraction technologies.

Phytovolatilization has been related to specific enzymes produced by rhizospheric microorganisms and therefore it should be better called plant-assisted biovolatlization.

Azaizeh et al. (1997) studied selenium volatilisation in the rhizosphere and bulk soil of a constructed wetland. They demonstrated that Se volatilization from rhizosphere soil cultures is generally higher than from bulk soil cultures. They concluded that microbial populations in the rhizosphere appear to be more effective in volatilizing Se than those present in non-rhizosphere soil.

3. Conclusions

While the fate of some micronutrients in the rhizosphere is well documented, there is substantial lack of information on contaminant metals and metalloids. Virtually nothing is known about metal - root interactions in the rhizosphere of metal hyperaccumulator plants. Future research should be directed to establish the role of rhizosphere processes in the various phytoremediation technologies on a metal-specific basis. A series of experiments are currently ongoing to carachterise the composition of root exudates of *T. caerulescens* and to investigate the influence of heavy metals. Other experiments aim to assess the ability of *T. caerulscens* to mobilize heavy metals (in particular Cd and Zn) in polluted soils.

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VARIATIONS OF RADIOCAESIUM BIOAVAILABILITY IN BULK AND RHIZOSPHERE SOILS AS INFLUENCED BY WILLOW PLANT GROWTH

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1. Introduction

In soils, radiocaesium is fixed by micaceous minerals. The potential contribution of these minerals in supplying K to higher plants is also important and relates to the possibility of enhanced weathering of mica in the rhizosphere. In laboratory and field conditions, evidence for mineralogical changes and intensified mica weathering occurring at the soil-root interface is now well documented (Hinsinger *et al.*, 1992; Courchesne and Gobran, 1997). Mica weathering induced by roots relates mainly to the depletion of K occurring around the most active part of the roots. This process results in a shift of the exchange equilibrium between available K and interlayer potassium of micaceous clay minerals. However, the possibility of extended mobilization of radiocaesium in the rhizospheric environment of plants due to enhanced weathering of mica has not yet been considered. This study will concentrate on what extent such mechanisms can affect the radiocaesium mobilisation by plant roots as a result of *Salix viminalis* cutting growth.

2. Materials and Methods

Experiments are carried out in simplified conditions from which composite samples of rhizospheric "environment" and bulk "soil" can be distinguished (Leyval, 1989). One year old *Salix viminalis* cuttings were planted in PVC cylinders (4.5 l) containing a mixture of water-washed quartz sand (500-1000 µm) and phlogopite minerals (100-200 µm) in the proportion 100g:1g. The micaceous minerals were previously equilibrated with two nutrient solutions containing or not potassium but similarly loaded with ^{134}CsCl. For each scenario, cylinders (2 replicates) were fed regularly with the respective nutrient solutions free of ^{134}Cs. After 3 months, K and ^{134}Cs content were determined in the plant and the leachates. The following determinations were made on both the rhizosphere and the bulk soil: total K and ^{134}Cs, exch. K and ^{134}Cs (NH_4Cl 0.1M) and XR Diffraction analysis.

3. Results an Discussion

The distribution of radiocaesium in different compartments (plant, leachates and soil) is illustrated in figure 1. In this figure, K and ^{134}Cs relative content as well as XR diffractograms performed on rhizospheric fraction are also presented.

Independently of the nutrient medium (containing or not potassium), the total amounts of radiocaesium mobilized both by the plant and the leachates are similarly low (< 0.6% of the total activity in the culture). In the K-free scenario, radiocaesium quantities mobilised by plant

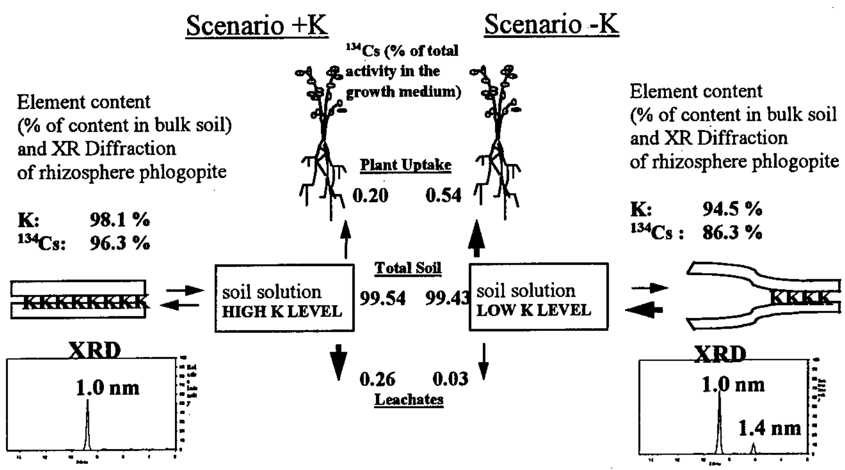

Figure 1: Schematic representation of the ^{134}Cs-soil-plant interactions

uptake are 2.5 times higher than those measured in the K-scenario. Moreover, plant uptake was also much higher than the losses of radiocaesium in collected leachates. Measurements of exchangeable ^{134}Cs amounts are however lower in the K-free scenario suggesting that non exchangeable elements would be removed under roots action. X-ray diffractograms obtained for "rhizospheric" phlogopite show a characteristic 1.0 nm reflection in both scenarios and an additional 1.4 spacing associated with the weathering of the mica only in the K-free scenario. In the former medium, the vermiculitization of the initial phlogopite is indeed connected with a lower total potassium content expressed in percent of the K content in the bulk fraction. The K depletion evidently induce a higher rhizospheric mobilization of radiocaesium.

At the soil level, our results show that in diluted potassium background solution, the plant roots are able to induce the removal of interlayer potassium and thereby a rapid mica weathering and an increased extraction of non exchangeable radiocaesium. At the plant level, they confirm that radiocaesium availability for plant can be simultaneously magnified due to a depressed competition at the soil solution-root interface.

4. Conclusions

The potassium depletion which prevails in the rhizosphere of plants was shown as an important process affecting simultaneously radiocaesium retention by micaceous components due to weathering effect as well as root uptake due to lower competition. This mechanism which is generally obscured by considering only the bulk soil could explain the long-lasting radiocaesium availability as observed in certain forest soils, permanent pasture or agricultural soil with a low potassium status.

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THE EFFECT OF DIFFERENT NITROGEN FORMS ON THE ACCUMULATION OF CD IN PLANTS

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1. Introduction

The form of nitrogen applied as plant nutrient can play an important role in the uptake of other elements. Plants grown on NH_4^+-N nutrient solution had a lower cation concentration compared to plants grown on NO_3^--N solution. The effect is diminished when plants are grown in the soil. The effect of application of ammonium sulphate, calcium nitrate and ammonium nitrate on the accumulation of Cd in plant biomass and their availability in soil was investigated in model pot experiment.

2. Materials and Methods

The experiment was conducted on acid luvisols (pH_{KCl} = 5.1, CEC = 23 mval.100g^{-1}, C_{ox}= 2.3 %) with total concentration of Cd = 0.9 mg.kg^{-1}. Five kg of soil was thoroughly mixed with 0.75 g N applied as solution of $(NH_4)_2SO_4$ - [AS], $Ca(NO_3)_2$ - [CaN], and NH_4NO_3 - [AN] in 20 ml of water. P and K were added as K_2HPO_4 at rate of 0.44 g P and 1.1 g K per pot in the same time. Spinach (var. Monores) was planted at four replication of each treatment and harvested in fully developed leaves. Soil was used subsequently in six growing cycles (three in spring and three in autumn). N,P,K were applied at the same rate before each growing cycle. Total Cd concentration in the dry matter of plants and the extractable pool of Cd released by three extractants (HNO_3, DTPA, and $CaCl_2$) were determined by AAS.

3. Results and Discussion

The application of different forms of nitrogen affected all investigated parameters in the most growing cycles. Spinach yield was affected by growing cycle and tended to be the highest with the CaN treatment. There were not large differences between the other two treatments only in one of six cycles was a significantly higher yield at AN treatment was found. Accumulation of Cd in plant biomass is shown in Fig. 1. AN treatment as a source of both forms of N showed higher Cd accumulation compared to CaN at all six cycles. In the first three cycles the difference was not as high as in the second three cycles, probably due to by the form of N used but due to past decreased in soil pH. The AS tended to higher accumulation of Cd in the biomass compared to the control treatment but the relationship was not clear.

The availability of Cd in soil was also determined by three extractants. The best correlation between soil extractable and plant Cd concentrations were determined if 0.01 mol.l^{-1} $CaCl_2$ was used. The lowest content of extractable Cd was found with CaN and the shape of the curve showed a similar pattern with Cd concentration in the plants (Fig.2). Significantly higher extractability of Cd in the AS treatment soil than in the AN one was not confirmed by different Cd accumulation in spinach biomass.

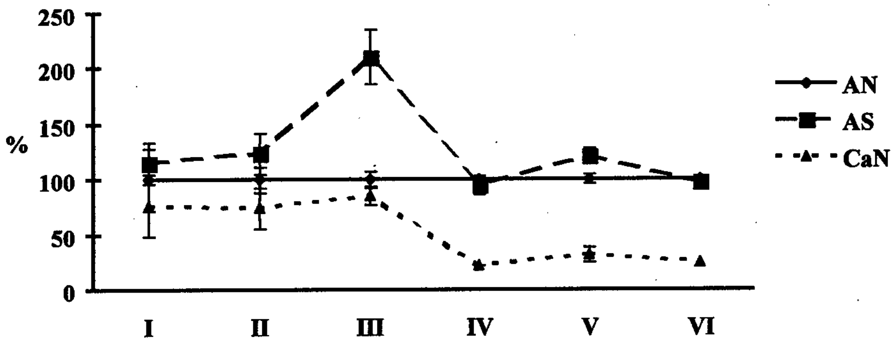

Fig. 1: The relative changes in Cd concentration (%) in spinach leaves in six growing cycles (AN - NH_4NO_3, AS - $(NH_4)_2SO_4$, CaN - $Ca(NO_3)_2$)

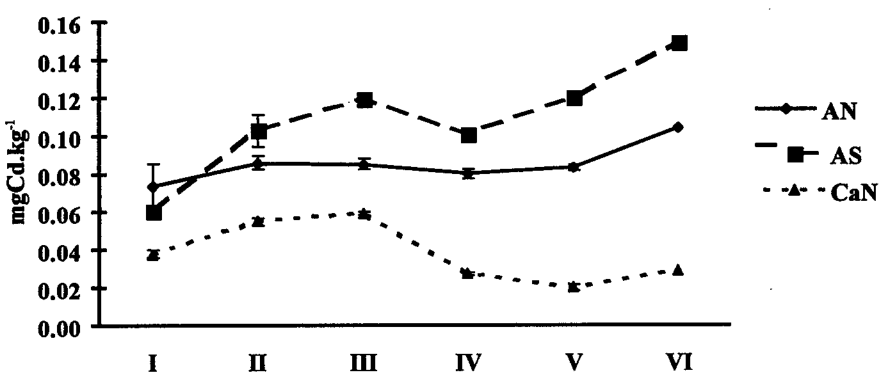

Fig. 2: The soil Cd release (mg.kg^{-1}) by 0.01 mol.l^{-1} $CaCl_2$ in six growing cycles

4. Conclusions

The form of nitrogen applied into the soil affected the yield, element content in spinach biomass and the extractability of Cd from the soil. The highest yield with the lowest Cd concentration was found with the calcium nitrate treatment and was confirmed by the lowest Cd extractability by 0.01 mol.l^{-1} $CaCl_2$.

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IMPACT OF NICKEL ON THE DISTRIBUTION OF CU AND ZN ACROSS THE RHIZOSPHERE OF WHEAT

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1. Introduction

One of the Prime needs of the agronomists is to measure environmental effects with the rhizosphere, since the soil conditions near the roots are different from those in the bulk soil (Nye and Tinker, 1978; Barber, 1984 and Youssef and Chino, 1991). So far, no success has been achieved experimentally in determing whether the effect of root processes in the rhizosphere soil may or may not be directly involved in the solubilization of heavy metals. In our rhizosphere research, it was observed that plant roots greatly affect soil pH in their vicinity, which in turn may affect heavy metals availability across the rhizosphere (Marschner and Romheld, 1983, Youssef and Chino, 1989; Youssef et al. 1999. The experiment designed to investigate the effect of adding S and/ or Ni on the concentration of Cu and Zn in the rhizosphere and in plant parts of wheat seedlings.

2. Materials and Methods

The rhizoboxes were filled with a soil Previously treated with fertilizer as follows: 0.5 Urea and 0.35 g $KH_2 PO_4$ /kg soil. Three levels of elemental S (S0 , S1, S2) were added, namely 0,1 and 2 g/kg soil, uniformly mixed with soil, and Ni was added at rate of 60 mg as $NiCl_2.6H_2O$/Kg soil. Treatments were namely, S0Ni0, S1Ni0, S2Ni0, S0Ni1, S2Ni1. Briefly, the rhizobox system consists of several soil compartments differing in thickness, and separated by 500 mesh nylon cloth. Wheat seedlings were planted in the central compartment (C.C.), experiemtnal techniques are described in more details elsewhere (Youssef, 1997).

3. Results and Discussion

The roots decreased the pH between 0.1 m 0.3 units across the rhizosphere being most pronounced in the C.C where the plant roots were grown (Table 1). Besides, S-application caused pH reduction less than 0.5 units and the lowest pH value was recorded in S1N1 treatment. Data were described in more details in Youssef (1997).

A series of analysis has been conducted to shed some light on the availability of Cu and Zn across the rhizosphere of wheat. Table 2 shows that S-application increased significantly the extractable Cu and Zn. The magnitude of this increase varied between Cu and Zn. Gupta and Macload (1980) reported that S-application enhanced the availability of micromutrients in different soils. The application of Ni alone to the soil decreased the extractable Cu and Zn in the C.C. by 17% and 28%, respectively compared to that of bulk soil. At S0Ni0 and S1Ni0 treatments, extractable Zn decreased toward the C.C. While, soil pH decreased in those treatments. Linehan et al. (1985) found that Cu and Zn increased in the rhizosphere of barley comparedf to that of bulk soil. They reported that the patterns of mobilization of Cu and Zn were parallel the patterns of changing soil biomass which might point in the direction of microbial involvement in heavy metals mobilization. However, both soil micribes and the influence of plant roots may be involved. The content and uptake of Cu and Zn in plant parts (shoots, roots) in relation to the soil treatments and metal concentrations at the different distances from root planes will be presented.

Table 1: Effect of soil treatments on pH changes across the rhizosphere of wheat seedlings.

Distance from (D) root (mm)	Soil treatments					
	S0N10	S1N10	S2N10	S0N11	S1N11	S2N11
	pH values (water)					
C.C	7.4	7.2	7.1	7.4	7.0	7.2
1	7.5	7.2	7.2	7.5	7.3	7.4
2	7.6	7.3	7.3	7.6	7.4	7.4
Bulk (>5mm)	7.6	7.3	7.4	7.6	7.3	7.5

Table 2: Effect of soil treatments on the availability of heavy metals (μg/g) soil across the rhizosphere of wheat seedlings.

Distance from (D) root (mm)	Soil treatments (T)						
	S0N10	S1N10	S2N10	S0N11	S1N11	S2N11	Mean
	Extractable Cu						
C.C	3.70	3.93	4.02	3.62	4.88	4.87	4.17
1	4.00	4.03	4.24	4.21	3.87	3.91	4.04
2	3.90	4.00	4.23	4.36	3.73	3.77	4.00
Bulk (>5mm)	3.98	4.00	3.97	4.38	3.89	3.92	4.02
Mean	3.90	4.00	4.12	4.14	4.09	4.12	
	LSD 5%		T=0.11	D = 0.07	TXD = 0.21		
	Extractable Zn						
C.C	15.08	17.76	31.48	14.92	26.12	24.92	21.71
1	18.00	19.60	21.08	18.40	24.12	26.92	21.35
2	18.80	19.20	18.92	20.80	20.52	21.32	19.93
Bulk (>5mm)	18.00	20.28	18.64	20.80	20.00	20.80	19.75
Mean	17.48	19.21	22.53	18.73	22.69	23.49	
LSD 5%		T= 1.20	D = 1.09	TXD = 2.35			

T: Treatment D: Distance

4. Conclusions

(1) The uptake of heavy metals (Cu, Zn) is greatly dependant on their concentrations in the rhizosphere, and to some extent to the bulk soil.

(2) The distribution of the metals at the soil root interface is more important for evaluation of the dynamics of heavy metals than their solubility in the bulk

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SURVIVAL AND ADAPTATION OF RHIZOBIAL POPULATION INOCULATED INTO INDUSTRIALLY CONTAMINATED SOILS

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1. Introduction

Industrial activities and the disposal of waste products have resulted in the contamination of many terrestrial environments. Industrial effuents often contain considerable amounts of potencially toxic metals such as Cu, Zn, Ni, Cd, Pb, Hg and Cr.

Experimental results published to date indicate that numbers of soil microorganisms, or their activities, are reduced in soils contaminated with heavy metals at concentrations close to those stipulated in the U.K.(McGrath *et al.*, 1988; Giller and McGrath, 1989).

For this study, we selected soils from an area with known pollution problems where heavy metals and other pollutants have been emitted by industry for nearly 40 y. This area is particulary affected by the release of liquid effluents from fertilizer industries and chemical industries. Previous work indicated a change in the genetic diversity of rhizobial population present in this contaminated soil. The aim of this work was to assess the survival of strains inoculated into the contaminated soil and to follow their behaviour over a 18 months period after inoculation.

2. Materials and Methods

We used soils from an arable field. One was collected near an industrial effluent channel contaminated by heavy metals (soil I) mainly Zn (375 mg kg^{-1}) and Hg (33 mg kg^{-1}). The other was of identical texture and similar organic matter and pH (Castro *et al.*, 1997), but not contaminated by heavy metals (soil IA). Soil samples were sterilized by Gamma-irradiation (25 kGy) and were inoculated, separately, with *Rhizobium leguminosarum* bv. *trifolii* strains (19 TsI, 23 TsI, 21 TsIA, 23 TsIA, 24 TsIA and 30 TsIA) previously isolated from root nodules of *Trifolium subterraneum*, L. grown in both soils. Soil I was inoculated with these six strains and soil IA, was inoculated only with two strains (19 Ts1 and 23 TsIA). After inoculation (with 10^7 cells), all soil samples were sealed and placed in the dark at 15 °C. Uninoculated soils were used as controls. Three, six, twelve and eighteen months, after inoculation, survival of rhizobia from each treatment (soil x strain) was estimated by the Most Probable Number (MPN) method, using subclover as host and a tenfold dilution series.

Twelve months after inoculation, subclover was also inoculated with soil suspensions from each treatment and 10 isolates, obtained from the nodules, were analysed for plasmid content using horizontal gel elecytrophoresis (Eckhardt, 1978).

3. Results and Discussion

No nodules were found in plants from uninoculated controls during the experiment, which confirms the efficiency of irradiation as a sterilant.

Three and six months after inoculation, the rhizobial population introduced into contaminated soils generally increased in all the treatments (10^8 bact. g^{-1} soil). Differences in growth rates became more visible 12 months after inoculation (Fig.1), showing that treatments inoculated

with 19 TsI and 23 TsI (both originally from contaminated soil) the highest growth rates among the *Rhizobium* strains. Only at 18 months was a decrease in the numbers observed. Treatments inoculated with 23 TsIA and 30 TsIA had the lowest number of bacteria g^{-1} soil, ($<10^7$) and 19 TsI and 23 TsI had the highest.

Plasmid profiles of isolates obtained from plant nodules, grown in treatments 19 TsI, 23 TsI, 23 TsIA e 30 TsIA from contaminated soils, analysed 12 months after inoculation, were always identical to the original. However isolates from 21 TsIA and 24 TsIA treatments, showed a change of 10 and 40%, respectively, in their plasmid profiles. This rearrangement could be seen as an adaptation of strains to the new environmental soil conditions, because plasmids can play an important role in the survival. This could be the reason why in these treatments, rhizobial population survived better, when compared with treatments 23 TsIA and 30 TsIA.

The rhizobial population introduced in uncontaminated soils were always $\geq 10^8$ bact.g^{-1} soil and no changes in the plasmids profiles were detected during the experiment.

This study demonstrates the need for long term experiment to observe effects of heavy metal toxicity on the bacteria. Differences in growth rates could be seen as a bacterial response to a metal stress. Delays in growth initiation and slower growth occur in sensitive organisms when mercury is present (Robinson and Touvinen, 1984) and we could expected also to occur with other metals. Knight and McGrath (1995), found that responses to environmentally relevant concentrations of Zn and Cd took the form of decreased growth rates of a sensitive isolate of *Rhizobium*, but not of a tolerant isolate.

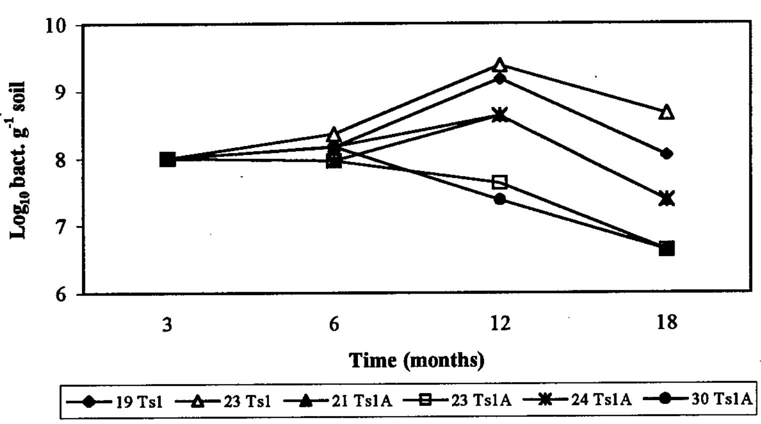

Fig.1. Growth rates of *Rhizobium leguminosarum* bv. *trifolii* srains inoculated in contaminated soils

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BIOAVAILABILITY OF COPPER IN THE RHIZOSPHERE OF RAPE AND RYEGRASS CROPPED IN VINEYARD SOILS

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1. Introduction

Copper has been applied in large amounts in vineyards over the last hundred years, because of the efficiency of copper salts (Bordeaux mixture) as fungicide against mildew. As a result of this agricultural practice, copper contents have been built up in vineyard soils, total copper contents commonly reaching several hundreds of mg kg^{-1} (Flores-Vélez et al., 1996). Only limited studies have been conducted in the Languedoc vineyard in the South of France (Brun et al., 1998).Over the last ten years, about a quarter of the surface area (100,000 out of over 400,000 ha) has shifted to other crops or land uses. The question arises now of the potential bioavailability of copper to crops and pasture species in such soils. In the present work, a bio-assay compared two plant species grown on two vineyard soils, either treated or not with a copper-contaminated sewage sludge.

2. Materials and Methods

The two topsoils were sampled in vineyards from Roquebrun (acidic lithosol) and Roujan (calcaric regosol), west of Montpellier (South of France). Their total copper content, as determined by an extraction with concentrated HF, amounted to, respectively, 53 and 175 mg kg^{-1}. The soil samples were incubated in wet conditions at 25°C for 5 month with or without 20 g kg^{-1} sewage sludge (heat-treated sludge containing 757 mg Cu kg^{-1}). The soils were then cropped for 14 days in contact with a dense mat of roots obtained with the technique described by Guivarch et al. (1998). This technique facilitates the collection of all the roots (and shoots) on the one hand, and the corresponding rhizosphere soil on the other hand. The whole cropped soil was used as such by the end of the experiment, being at less than 3 mm from the mat of roots in the culture device. Two species were compared: oilseed rape (*Brassica napus* cv Goeland) and perennial ryegrass (*Lolium perenne* cv Aubisque). The concentrations of copper in plant parts and the amounts of copper that accumulated in the roots and the shoots were then determined after wet digestion with a mixture of concentrated $HN0_3$ and $HClO_4$. They were compared with the amounts of total copper in the soil and with the amounts of copper extracted by 0.05*M* $CaCl_2$, DTPA, EDTA and 1*M* HCl from the soil before (control) or after cropping (rhizosphere). All the analyses of Cu were performed with flame atomic absorption spectrometry.

3. Results and Discussions

Little plant growth occurred in the soils without sludge, compared with the soils with sewage sludge. This was most likely the P-deficiency in the untreated vineyard soils. A very significant uptake of copper occurred in both plant species, the largest values being found for ryegrass and for Roujan site. A reduced uptake of copper was found for both species, as a response to the application of sewage sludge, especially for Roquebrun site. Another possible explanation for the increased growth of plants in response to sewage sludge application can be: adverse growth conditions related to excess copper in the soil might have been partly alleviated due to copper immobilization by sludge organic matter. A larger proportion of copper taken up by rape plants was translocated to the shoots, compared with ryegrass. The highest concentrations were systematically found in the roots: for Roujan site, they

ranged from about 30-50 μg g^{-1} DW for rape up to about 80-210 μg g^{-1} DW for ryegrass. This site yielded the highest contamination of plant parts. Concentrations in the shoots were close to reported phytotoxic values for rape (16 μg g^{-1} DW, Reuter and Robinson, 1997). For ryegrass the Cu concentrations were inadequate for sheep forage (10 μg g^{-1} DW, Coullery, 1996). The amounts of Cu extracted from the soil by 0.05*M* $CaCl_2$, DTPA, EDTA and 1*M* HCl ranked similarly as the amounts of Cu accumulated in the shoots or the whole plants (Table 1). In most cases, the amounts of Cu extracted with these various extractants significantly changed in the rhizosphere, relative to the control. The general trend found for ryegrass was a depletion of extractable Cu in the rhizosphere, whereas both increases and decreases occurred for rape, depending on the soil and the extractant. However, there was no quantitative relation between the amounts of extractable Cu depleted from the rhizosphere and the amounts of Cu taken up by both plant species.

4. Conclusions

The present work suggests that, even in calcareous soils where it is usually assumed that copper would be unavailable to plants, contamination of crops and pasture plants can be significant in vineyard soils. Further work is needed to confirm the results deduced from the controlled, specific conditions of the present bio-assay compared with levels of contamination measured *in situ*. Although, the precise mechanisms need to be elucidated, these results also suggest that the changes in copper speciation that can occur in the rhizosphere are a key factor of the ultimate bioavailability of copper in vineyard soils.

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Table 1. Amounts of Cu extracted by the plants and accumulated in the shoots or the whole plants (shoots + roots) and amounts of Cu extracted by the chemical extractants (all data expressed as μg Cu g^{-1} soil).

	Roquebrun	Roquebrun + sludge	Roujan	Roujan + sludge
Shoots				
Rape	0.06 ± 0.03	0.33 ± 0.08	0.52 ± 0.13	0.83 ± 0.15
Ryegrass	0.08 ± 0.08	0.30 ± 0.07	0.67 ± 0.11	0.94 ± 0.02
Shoots + Roots				
Rape	0.27 ± 0.08	0.58 ± 0.13	1.41 ± 0.06	1.71 ± 0.14
Ryegrass	0.18 ± 0.05	0.97 ± 0.20	2.14 ± 0.99	2.80 ± 0.53
EDTA	7.18 ± 0.09	16.04 ± 1.78	79.82 ± 2.82	92.78 ± 8.84
DTPA	3.38 ± 0.01	9.06 ± 0.29	61.21 ± 3.29	67.92 ± 8.06
$CaCl_2$	0.41 ± 0.00	0.20 ± 0.00	0.65 ± 0.00	1.16 ± 0.00

A TECHNIQUE FOR QUANTITATIVE TRACE ELEMENT AND MICRONUTRIENT STUDIES OF PLANTS

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1. Introduction

Traditionally when cultivating plants in the laboratory, there is a maintenance of a constant nutrient concentration in the rooting medium. External nutrient concentrations are held constant by balancing the rate of addition against the rate of nutrient removal by the plants. However, the maintenance of low concentrations about the root surface may be problematic and rapid flow or stirring of the culture solution is essential (Asher and Edwards 1983). A technique to apply nutrients to plants in balanced proportions and in amount that meets the requirement for plant growth has been used extensively in studies of plant nutrition during recent years (Ingestad 1982, McDonald *et al.* 1991, Ingestad and Ågren 1992). The approach eliminates the requirement of maintaining extremely low concentrations and involves the addition of nutrients at predetermined, exponentially increasing amounts to the culture solution. This is the 'steady-state' approach and the use of the relative addition rate, R_A (day^{-1}) of nutrients as the growth-controlling variable has shown that plant uptake of nutrients and growth can be strictly controlled during the exponential phase of growth. The steady-state approach has the advantage of eliminating the unintentional occurrence of nutrient deficiency, which can otherwise be a problem in interpreting growth responses in plant nutrition research. This is crucial when experimenting with potentially toxic elements (Göransson and Eldhuset 1995, Ericsson *et al.* 1998). The other main advantage with the approach is that well-defined levels of nutrient limitation can be maintained during the experimental period. Thus, growth response to an environmental variable may be studied in plants at different degrees of nutrient limitation. In the studies of the plant requirement of micro elements (Fe, Mn, Zn, Cu, B) (Göransson 1999, Göransson and McDonald 1993), a variety of plant species have been used. Inorganic salts are normally used in the added culture solutions. However, chalets may also be used.

2. Material and Methods

The relative addition of nutrients to the roots may be defined as the addition rate, *dn/dt* (g day^{-1}), with respect to the amount of nutrient, n (g), in the plant. By using R_A to control the relative uptake rate of nutrients, steady-state response, *i.e.* stable internal nutrient concentrations, *n/W*, and stable relative growth rates, R_G, may be attained. At steady-state nutrition, $R_A = R_G$ and is defined as:

$$R_A = \frac{1}{n}\frac{dn}{dt} = \frac{1}{W}\frac{dW}{dt} = R_G$$

where W (g) is the plant mass. At steady-state growth, the internal or plant nutrient concentration remains constant for a given genotype, growth environment and the chosen value of R_A (Ågren 1985):

$$\frac{d(\frac{n}{W})}{dt} = 0$$

When dealing with trace elements that may become toxic, and micronutrients that are required in diminutive amounts, one of the most important methodological considerations is a thorough

cleaning procedure of laboratory equipment, including the growth units. Small plants were then grown in the units in a culture solution free of the specific micronutrient that was to be used as the driving growth-variable in the following experiments. After one week, these plants were discarded and the growth unit was rinsed with distilled water. After a development of the method it has been possible to grow plants with approximate stable properties during the whole growth-season in the field (Ingestad 1988). The influx of nutrients to the plants then depends on external variables, *e.g.* light and temperature. A complete nutrient mixture is dissolved and injected into the irrigation water.

3. Results and Discussion

A strong casual relationship between the R_A of a growth-limiting micronutrient, plant micronutrient concentration, and the R_G of the plants was found to be general for all limiting micronutrient elements. The relationship between the internal concentration of the growth-limiting element and the R_G was linear and positive. The method has been used to make models for nutrient uptake in plants (Ingestad 1988) and to model the inhibiting effects of Al on Ca and Mg uptake (van Oene 1998). Results from laboratory studies have been used to calculate the micronutrient dosage in field trials with *Picea abies* (Linder 1995). All the results found in the laboratory have been demonstrated to be relevant in the field trials. The findings have illustrated the feasibility and usefulness of the 'steady-state' approach in plant nutrition to studies of potentially toxic substances and growth response.

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OXIDATIVE STRESS - BIOCHEMICAL BASE FOR ITS COPPER-DEPENDENCE IN LUPIN AND SOYBEAN NODULES

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1. Introduction

In some symbioses the nitrogen fixation and legume yields may be limited by low availability of copper Cu, but the direct biochemical role of Cu in nodule function is not yet clear. The author's previous work have shown that in the pot experiment conditions increasing leghemoglobin concentration and nitrogenase activity in root nodules and increasing nitrogen accumulation in plants were sensitive responses of N_2 fixation to Cu nutrition in yellow lupin but not in soybean (Seliga H. 1993, 1998). In order to investigate differential sensitivity of grain legume N_2 fixation on low availability of Cu, it seemed advisable to pay attention to the action of Cu deficiency in legume nodules in context of oxidative stress. In the last decade it has become clear that in humans and animals, oxidative stress underlies many diseases and contributes to the process of aging. Plant studies in the field are less abundant but negative effects of oxidative stress due to the formation of reactive oxygen species are still stronger in plant than animal cells because of higher oxygen concentration in tissues and the presence of light photoreceptors. Oxidative stress, defined as a shift of the balance between prooxidative and antioxidative reactions in favour of the former is presently believed to be a common denominator of the action of various environmental and biological factors. One of many elicitors of oxidative stress may be the levels of transition metal ions (Caro A. and Puntarulo S. 1996).

The aim of present study was to analyse the effect of Cu nutrition on nodule oxidative processes. In this paper the effect of Cu supply on lipid peroxidation and the level of total phenol in nodules and on the interaction with iron in two grain legume species with contrasting sensitivity to Cu nutrition was determined.

2. Materials and Methods

Lupinus luteus L. cv. "Juno" and *Glicine max.* L. cv. "Progres" inoculated with *Bradyrhizobium* strains, were grown in a greenhouse in pots on Cu deficient peat soil, mixed with sand at two Cu levels: 0 (withouth Cu - Cu_0) and 125 mg Cu per pot (optimum - Cu_{125}) and fed with basic nutrient solution without nitrogen . Plant material (dry matter) was analysed for Cu and Fe content by atomic absorption spectrometry. For the measurement of lipid peroxidation in nodules, the thiobarbituric acid (TBA) assay which determines malonodialdehyde (MDA) as an end product of lipid peroxidation was used. Total phenol concentration was determined in ethanol solution with the Hathway reagent according to the prussian blue methods.

3. Results and Discussion

The accumulation of dry matter and nitrogen in the whole plants , nodulation and N concentration in nodules were significantly increased by Cu supply at the flowering stage in yellow lupin plants but not in soybean plants. As it was shown previously, these effects could be related to the function of Cu in the N_2 fixation process (Seliga H. 1998). However Cu nutrition significantly increased the Cu concentration in nodules of both tested plants .This suggests that requirements of Cu by symbiotic N_2 fixation in nodules of yellow lupin was higher than in

nodules of soybean. Yellow lupin plants more sensitive to Cu deficiency showed the higher quantity of phenol in nodules and the Cu supply significantly decreased phenol concentration in nodules of these plants at early growth stages. At the same time about 2-fold difference in nodule lipid peroxidation between the Cu_o and the Cu_{125} lupin plants was observed. In contrast to soybean nodules Cu deficiency in yellow lupin nodules caused an increase of lipid peroxidation. The highest enhancement of nodule lipid peroxidation was recorded at 21-31 days after sowing and it decreased by about 100% from day 44 in the Cu_o and Cu_{125} in both tested plants. Thus, the results showing the differentation of lipid peroxidation to Cu nutrition in tested grain legume species could be related to the level of phenols in nodules and dependent on the both host plants and strains of rhizobia, which differ in their ability to produce catechol-like siderophores. There are to our knowledge, no previous reports of siderophores formed in symbiotic association between rhizobia and plants (Wittenberg J.B at al. 1996). Copper may effectively participate in oxidative reactions of nodule phenolic compounds. Phenols chemical assays are used as test for the measurement of catechol-like siderophores, which are iron-binding chelators and they are able to acquire iron more effectively. In the present experiments it was found that Cu deficiency in soil limited iron uptake and its translocation to the nodules only in lupin plants. Moreover significant differences were found between more Cu sensitive yellow lupin and less sensitive soybean plants tested for nodule phenol:Fe ratio, similary to phenol-Cu ratio. This suggests that a high phenol content in yellow lupin nodules, in contrast with soybean could be important factor in the Cu-phenol interaction which could play a role in iron accumulation and reactivity. Therefore, the Cu nutrition could have a protective effect on iron-induced lipid peroxidation in lupin nodules as ferrous ions are known to be a strong stimulators of lipid peroxidation in living organisms .

4. Conclusions

Grain legume differ considerably in sensitivity of N_2 fixation in their nodules to Cu nutrition. In the experiments reported here, the strongly inhibited lipid peroxidation as result of Cu treatment indicate that there is the patological significanc of the action of Cu deficiency on generation of free radicals and increased lipid peroxidation in yellow lupin nodules. In this paper the biochemical basis for Cu-dependent oxidative stress occurence due to the formation of reactive oxygen species in nodules of tested grain legume species is discussed. The results obtained suggest that antioxidative activity of Cu in effective protection against oxidative stress in grain legume nodules could be connected with the level of nodule catechol-like siderophores and iron metabolism.

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MICRONUTRIENTS UPTAKE IN RED BEET (*BETA VULGARIS* L.) INOCULATED WITH PLANT GROWTH PROMOTING RHIZOBACTERIA OF THE *PSEUDOMONAS* GENUS ON VARIOUS PRODUCTIVE SOILS

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1. Introduction

Specific strains of bacteria of the *Pseudomonas* genus have been shown, when incorporated into rhizosphere, to increase the yield of some agricultural crops (Şuslov and Schroth 1982; Hofte et al. 1991). One of the suggested mechanisms of plant growth stimulation by bacteria includes an improvement of nutrients uptake in plants (Meyer and Linderman 1986; Mineev et al. 1991). Little research has been undertaken on trace elements uptake in plants and the response of non-legumes to inoculation with plant growth promoting pseudomonads in dependence on different soil conditions. In the present study different soils and artificial substrate mixtures with a great variety of physicochemical properties were examined to evaluate the uptake of Fe, Zn, Mn, Cu, Co and Mo in red beet and the influence of seeds inoculation with plant growth promoting bacterium of the *Pseudomonas* genus on this process and plant growth response.

2. Materials and Methods

Red beet plants (*Beta* vulgaris L.) were grown in polyvinyl chloride pots (0.33x0.33x0.33m) without bottoms buried in soil and filled with soils and substrates of increasing fertility levels and supplied with NPK fertilizers at the rates of 1.2 g pot^{-1}. Seeds were inoculated with 10^7 colony forming units of bacterium. In the experiments gray forest soil:sand (1:1 v/v), soddy podsolic soil, gray forest soil, valley soil, valley soil:manure have been used. Soil moisture in pots was maintained above 60% WHC.

3. Results and Discussion

The plant weight, concentration and uptake of trace elements by yield showed large differences between the soils and substrates. Root weight rose with the increase of soil fertility level and reached maximum on the productive valley soil. Content of Fe in root and shoot was the highest in gray forest soil and in valley soil with and without the addition of manure. In contrast, Mn concentration in plant was minimum in the mixture of gray forest soil with sand and in high productive soils. In general, content of other elements in plants differed insignificantly.

Inoculation of plants with bacterium in poor soils enhanced the yield of root and shoot by 10-40% and 17-55% respectively, but increased only shoot weight in valley soil and in valley soil with manure by 53 and 38% respectively. Introduction of bacterium into the rhizosphere of red beet led to a considerable increase of root concentration of Zn in all soils, Cu and Co in soils with high productive level, Mn in both mixed substrates, Fe in soddy podsolic soil and in both substrates. On the contrary, in the treatment with bacterium the decrease in root of the contents of Mn in valley soil and Mo in all soils except the mixture of valley soil with manure was found. The bacterium did not considerably influence the concentration of most of the elements in shoot. Inoculation significantly ($P<0.05$) increased the uptake by the total yield of all elements except Mo in all soils and Mn in valley soil:manure (Table). Accumulation of most of the elements in inoculated plants was greater in soils with high productive level as well as in uninoculated plants.

Table. Elements uptake in yield of red beet

Soils	Treatment	Fe	Zn	Mn	Cu	Co	Mo
		(mg pot^{-1})			(μg pot^{-1})		
Gray forest:sand	Uninoculated control	58	8	31	901	553	34
	Pseudomonas	88	15	46	1289	1000	32
Soddy podsolic	Uninoculated control	57	13	61	1003	589	28
	Pseudomonas	77	19	70	1254	812	29
Gray forest	Uninoculated control	108	13	60	1095	699	35
	Pseudomonas	122	17	68	1619	996	35
Valley	Uninoculated control	119	13	13	1167	733	36
	Pseudomonas	139	23	21	1834	1062	38
Valley:manure	Uninoculated control	121	15	20	1075	917	42
	Pseudomonas	172	23	21	1929	1093	43

There are two possible explanations for the greater uptake of elements by plants inoculated with *Pseudomonas*. First, bacterium can improve elements uptake in plant as a result of solubilization of unavailable forms. It is known that pseudomonads solubilize unavailable forms of phosphorus (Hoflich 1992). Secondly, the roots of inoculated plants can take up more elements than uninoculated roots. In our greenhouse assay with split-root system of corn (*Zea mays* L.) it was shown that pseudomonads having no contact with soil enhanced the ability of roots to uptake mineral elements from soil (Shabayev et al 1999). However, maximum effect of bacterium to increase the uptake of elements in plants was found when bacterium was added to soil.

4. Conclusions

The results of this study indicate that nutritional status of soil influences trace elements uptake in plants and their response to inoculation with bacterium. Root yield of red beet and accumulation of most of elements in plants were highest on great productive soil and substrate. Inoculation of red beet with *Pseudomonas* considerably promotes plant growth and increases the uptake of most of elements by total biomass independent of soil fertility level. Soils and substrate low in fertility level showed the highest root yield response to inoculation with bacterium.

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THE UPTAKE PROPERTIES OF POTASSIUM INTHE RHIZOSPHERE OF DIFFERENT SUGARBEET GENOTYPES

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1. Introduction

The results from two experiments conducted with different sugarbeet variety and soil environment are shown and the potassium uptake characteristics in the rhizosphere of sugarbeet is described.

2. Materials and Methods

The experiment included two minipot trials with two sugarbeet varieties (Elan,Colibri),two fertilizer rates (nofertilizer, fertilized with 100 mg kg^{-1} N, P_2O_5 and K_2O) and two sodium and potassium fertilizer rates(100mg/kg Na, Na+K, K). All mini-pots were filled with a subsoil containing 210 g kg^{-1} water and a bulk dencity adjusted to 1.7 g cm^{-3} . 280 pregerminated sugarbeet seeds were evenly placed in each pot and grown in the condition of daytime (light intensity 200 $\mu molm^{-2}s^{-1}$,16h, 20^0C) and night (8h, 10°C).

3. Results and Discussion

Sugarbeet seedlings strongly depleted soil K in the vicinity of their roots forming a K depletion profile. To describe the K uptake characteristics in the rhizosphere, the mathmetical function $y=a(1-e^{-(b+cx)})$, b>0, c>0, was fitted to the depletion profile of the sugarbeet rhizosphere. Although the sugarbeet genotype and the soil fertility were different, all regression curves gave reasonable fits (see table 1).

The K uptake ability of variety Elan is notably higher than that of variety Colibri. The depletion range, depleted soil volume per unit root and the amount of total exchangable K taken up by the two varieties are reported in Table 1.

Fertilization has a great influence on K uptake profiles in the sugar beet rhizosphere. The range of depletion, depleted soil volume and the amount of K taken up in the fertilized treatment by variety Elan is 2.6, 6.9, 5.2 times larger than in the untreated soil, respectively. For the variety Colibri sodium application had a different effect on K uptake by these sugar beet genotypes. At adiquate soil K concentrations, Na treatment tended to decrease K uptake by the variety Elan, but enhanced the K uptake ability of variety Colibri (see Table 1).

Table 1. The properties of K uptake under different fertiliser conditions in the rhizosphere of two different sugarbeet genotypes

Trial Nr.	Fertilizer	Variety	Coeff. determination R2	Depleted range mm	depleted soil volume cm^3/cm root	K taken up by plant mg/pot
1	No fertilizer	Elan	0.82	9.4(0.4)	2.78(0.15)	0.96(0.05)
		Colibri	0.89	8.8(0.3)	2.43(0.12)	0.82(0.04)
	Fertilized	Elan	0.96	24.7(0.5)	19.17(0.9)	4.99(0.07)
		Colibri	0.97	19.3(0.6)	11.7(0.23)	4.32(0.08)
2	Na	Elan	0.95	10.0(0.6)	3.14(0.21)	1.21(0.35)
	K+Na	Elan	0.99	12.2(0.4)	4.68(0.17)	3.23(0.21)
	K	Elan	0.99	13.0(0.3)	5.31(0.1)	3.63(0.18)
	Na	Colibri	0.94	9.4(0.3)	2.77(0.24)	1.00(0.12)
	K+Na	Colibri	0.99	13.0(0.6)	5.31(0.48)	3.45(0.24)
	K	Colibri	0.99	11.6(0.4)	4.23(0.32)	3.00(0.17)

* The number in brackets denotes standard error

4. Conclusions

Sugarbeet seedlings can strongly deplet soil exchangable K and form a depletion profile which can be well described by $Y=a[1-e^{-(b+cx)}]$. The K uptake and the K depletion zone in the rhizosphere are varying with cultivar, time of cultivation and fertilizers. This study represents an example of differential distribution of an element in the rizosphere that may similarely be found for trace elements.

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Remediation of Metal -Contaminated Soils

(Special Symposium 6)

SAMPLING, ASSESSMENT AND RISK ANALYSES OF METAL CONTAMINATED SOILS

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1. Introduction

Information gained from the sampling of contaminated sites will be used to decide whether site remediation is necessary. If remediation is required, the information gained from the sampling will be used to decide what type of remediation is required.
Four distinct aims of the sampling are

- Detecting contamination on the site;
- Quantifying the extent and concentration of the contamination;
- Assessing whether the contamination poses an environmental risk;
- Assessing with the contamination poses a health risk.

The sampling strategy should be formulated to assist in the decision making process.
Historic and circumstantial information about the site contributes to the choice of sampling strategy chosen.
The type and intensity of sampling will also be affected by the proposed land use. For example the same level of contamination would pose less threat to commercial land that is to be covered by buildings or car parks than it would to residential land.

2. Detection of Contamination

An established method of sampling sites is to use a square grid. A triangular grid can give better coverage, but the advantage may be outweighed by the added complexity (especially at the boundaries of the area). Random sampling is not recommended.
Stratification of the site may give significant advantages. Generally the sampling intensity will differ between the strata. Stratification may be based on historical data or on local criteria.
Composite samples are recommended when the sampling is aimed at detecting local contamination. Compositing is particularly useful when the cost of collecting additional field samples is less than the cost of the chemical analysis. For example, compositing four surface samples can effectively halve the grid size for little extra expense. Compositing can also be effectively used at a 1m scale so each sample effectively represents several square metres (Ramsey *et al.*1995). A discussion of composite sampling is given by Patil *et al.* (1996).

3. Delineation of contaminated area

While composite samples are useful in the detection of a contaminated area, they are of limited use in delineating it. The delineation requires additional sampling, with each sample being individually analysed. Following the secondary sampling, interpolation is required to delineate the area that exceeds the 'safe' level. A comparison of interpolation methods can be found in Brus *et al.* (1996).

4. Sampling depth

There is little agreement on the depth to which a soil should be sampled. The depth of samples must be chosen so that it gives information that is useful for the decision making process. For example if an area is for recreational use only, the presence of non-mobile contaminants a meter below the surface is of little importance but surface contamination is very important, particularly in a children's playground..

In some cases the contaminant may be concentrated in the top 2-3 cms. There are real practical difficulties in obtaining accurate samples that represent that layer, so in practice surface samples representing 0-10cm are used.

5. How many samples are needed?

The number of samples required is a vexed question. 'Sufficient samples' may cost more than the value of the land. The number of samples required will be a function of the type of contaminant, the like and safe limits of the contaminant, the proposed land use, and the manner the contaminant was acquired. For example, few samples would be required for a diffuse source of contamination such as dust coming out a stack on to a field. By contrast, a high sampling intensity would be required if the contamination source was land fill, with only occasional small loads being contaminated.

The proportion of land that is contaminated is a special case – the number of samples required for a given degree of accuracy can be readily assessed.

6. Sample collection and preparation

The need to use non-contaminating implements has long been recognised. The appropriate type of auger (or perhaps spade) depends on soil conditions. In all cases the sample must contain a uniform amount of soil over the sampling interval.

Sample labelling is of the utmost importance. Double bagging with the soil in an inner bag and the label protected in the outer bag is a good approach.

Samples are often ground to pass through a sieve before analysis. The exact procedure needs to be carefully documented as there are differences between standards (including sieve types). Discarding a coarse fraction could create a bias - again the purpose of the sampling scheme must be considered before deciding what is apt.

7. Conclusion

Sampling contaminated sites can only be optimised when the purpose is understood.

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EX-SITU REMEDIATION OPTIONS AND FUNDAMENTAL PRINCIPLES

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1. Introduction

During the last decade various techniques for remediation of soils polluted with organic compounds have been developed and successfully applied in practice. With most of these techniques the pollutants are chemically or biologically destroyed. Soils polluted with heavy metals are much more difficult to remediate. Heavy metals can not be destroyed and their physical/chemical appearance in soil can vary significantly. This variety in physical/chemical states together with the variety in soil composition makes it difficult to develop universally applicable treatment techniques for these type of pollutants. Each polluted site is in fact different and requires a tailor-made solution. Cost effective techniques for removal of heavy metals or for immobilisation of heavy metals from soil are, therefore, still rather scarce. This paper will give an overview of the remediation options for ex-situ treatment of soil polluted with heavy metals.

2. Fundamental remediation principles

A clean-up technique is always based on one or more fundamental removal principles. A removal principle makes use of differences in properties between the pollutants or polluted particles and the other soil substances. Properties on which a technique for removal of heavy metals can be based are: the volatility of a pollutant (in the case of mercury); the solubility or leachability of a pollutant in a liquid phase; the electrical properties of a pollutant; the bioleachability of the pollutant; the tendency of a pollutant to hyperaccumulate in plants; the magnetic properties of a pollutant; the size, shape or density of a (particulate) pollutant.
Remediation of a contaminated site also can be obtained by a reduction of the heavy metal mobility in the soil. Such a reduction is primarily based on a chemical or physical transformation of the polluted soil.

3. Options for ex-situ treatment

Based on these fundamental remediation principles, various options for ex-situ and in-situ remediation of soil contaminated with heavy metals are available. The most important remediation options for ex-situ treatment of soil are: wet classification, chemical extraction, bioleaching, electroreclamation, thermal treatment, vitrification and physical/chemical stabilisation.

- Wet classification. This technique is aimed at the selective removal of heavy metal containing particles from the non-polluted soil particles. The process makes use of an aqueous phase to which one or more additives have been supplemented. Process steps to achieve this separation are sieving, screening, hydrocyclone treatment, magnetic separation, attrition and spiral classification.

- Chemical extraction. This process involves dissolving the heavy metals, present as particles or adsorbed onto the soil matrix, into an aqueous solution containing strong mineral acids
- (such as hydrochloric acid), chelating agents or surfactants. After separation of the soil, the liquid phase can be treated to concentrate the heavy metals.
- Bioleaching. In bioleaching the heavy metals in the soil are mobilised by acidophylic bacteria of the genus Thiobacillus which oxidise reduced sulphur compounds to sulphuric acid, increasing the solubility of the heavy metals. If reduced sulphur is not present an external reduced sulphur source has to be added to the soil. After separation of the treated soil and the metal sulphates containing liquid phase the latter can be treated to concentrate the heavy metals.
- Electrokinetic treatment. This is a clean-up method based on the application of an electric current between a cathode and anode placed in the soil. As a result of this electric current heavy metal ions and electrically charged small colloidal particles are transported through the water phase in the soil to the electrodes where they are concentrated and removed.
- Thermal treatment. This method is aimed at removal of pollutants by heating the soil to a temperature sufficiently high to vaporise the pollutants. The vaporised gas phase with the pollutants has to be treated in a subsequent treatment step. With regards to heavy metals, only mercury and mercury compounds can be removed from soil by thermal treatment.
- Vitrification. This is a treatment process in which the soil is heated to such a high temperature that the soil melts and the heavy metals present are incorporated in a glassy structure. The melt is formed under oxidising conditions promoting the conversion of heavy metals to oxides, silicates, etc. resulting in a strong reduction in leachability of the heavy metals.
- Physical/chemical immobilisation and stabilisation. The aim of these treatment methods is to decrease the leachability of the heavy metals by physical/chemical processes such as reduction, oxidation or encapsulation of the heavy metals in a matrix. Various treatment agents may be supplemented to produce favourable conditions for such an immobilisation.

4. Conclusions

Several modifications of the above mentioned options have been developed for practical treatment. Stage of development, field of application and practical experiences vary significantly. The most experience has been obtained with wet classification, vitrification and physical/chemical immobilisation. Critical issues for application are whether the technology can be implement at reasonable costs within a required time frame and at the desired level of treatment. There is a strong challenge for further improvement of existing techniques and development of new innovative techniques. In the paper, all these aspects are discussed in more detail.

IN SITU REMEDIATION OF METAL CONTAMINATED SOILS - OPTIONS AND FUNDAMENTAL PRINCIPLES

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1. Introduction

Remediation methods currently used to treat soils contaminated with heavy metals are expensive, labor - intensive and environmentally invasive. They are mainly based on civil-engineering techniques often involving excavation and removal of contaminated soil, with additional effort, time and money required to restore the site. A low - cost remediation technique that is environmentally sound, and equally protective of human health and the environment, would be a valuable and viable alternative to current remediation methods. Research has been directed towards exploiting biological and chemical processes to reduce the risk associated with metal contaminated soils.

The use of soil amendments and plants to alter the chemical and physical form of heavy metal contaminants in the environment, thus decreasing chemical and biological activity, shows promise. Inactivation, whether or not combined with phytostabilization, is for stabilizing trace elements that are potentially toxic. This should lresult to an attenuation of the impact on site and adjacent ecosystems. The main objective of *in - situ* immobilization is to decrease the risk of contaminant impact on the environment and human health. Effective and durable immobilization of metals reduces leaching and bioavailability. Subsequently, vegetation can develop to physically stabilize the soil. A new ecosystem can develop. This not only renders the site aesthetically pleasing but the vegetation cover further provides pollution control and stability to the soil. Lateral wind erosion and metal percolation to the ground water are greatly reduced.

The fundamental premise of many *in situ* remediation techniques is to reduce the relative bioavailability of metals in the soil by addition of soil amendments followed by subsequent establishment of biological communities which further immobilize soil metals. 'Bioavailability' is defined as metal fraction that can interact with a biological target. For soils, this depends on the physical and chemical characteristics of the matrix, metal speciation, and the target organisms.

Several field experiments have been performed in the NE of Belgium were several zinc smelters were in operation since the end of the former century. Results will be presented from both heavily contaminated old industrial sites and moderately contaminated kitchen gardens located near this smelter site.

2. Results and Discussion

In 1990, 3 ha of a metal contaminated acid sandy soil at the site of a former pyrometallurgical zinc smelter in Lommel-Maatheide (Belgium) were treated with a combination of cyclonic ashes ("beringite") and compost (Vangronsveld et al. 1995). After soil treatment and sowing of a mixture of metal tolerant *Agrostis capillaris* and *Festuca rubra* (both manipulations using conventional agricultural techniques), a healthy vegetation cover developed. Five and seven years later, evaluations were made of soil physico-chemical parameters, potential phytotoxicity, floristic and fungal diversity and mycorrhizal infection of the plant community (Vangronsveld et al. 1996).

Comparison of physico-chemical parameters of the non-treated and treated soils 5 years after application of the soil additive confirms and explains the absence of phytotoxicity: pH became slightly alkaline, organic matter content and cation exchange capacity increased and the water
extractable Zn fraction was up to 70 times smaller in the cyclonic ashes treated soil. Seven years after addition of soil amendments, vegetation was still healthy and regenerating both vegetatively and by seed. Diversity of higher plant species and saprophytic fungi was extremely low in the untreated area due to the metal toxicity and the absence of metal tolerant ecotypes of plants and fungi. On the treated soil, in contrast, there was greater diversity of higher plants (34 species, including woody species like birch, in 1998); several perennial forbs that are not noted as metal tolerant had colonized the revegetated area. Most of these species belong to mycotrophic families so that the presence of a mycorrhizal network in the soil promotes their establishment. Mycorrhizal fungi were ubiquitous in the roots showing that a functioning ecosystem was establishing. In non-treated soil, mycorrhizal infection rates of the roots were consistently lower during the growing season. Total number of bacteria in the treated area was shown to be 100-1000 times larger compared to the non-treated area. Moreover, preliminary results suggest a loss of tolerance against several metals. The total number of nematodes was about 4000 individuals per 100 g of soil, which is a normal value for sandy soils, compared to only a few individuals per 100 g soil on the non-treated soil. However, species diversity of nematodes on the treated soil still was very low; this possibly can be explained by the fact that the 3 ha test plot is situated in the middle of 135 ha of extremely toxic and completely bare soil, which can retard recolonization by metal sensitive species.
Ten kitchen gardens with sandy soils contaminated by aerial deposition from the same pyrometallurgical zinc smelter were treated with cyclonic ashes (1 ton/) use t $ha^{-1.}$ During the first year of experiments, gardens were partly treated with cyclonic ashes (5 %, by rototilling until a depth of about 25 cm). The same vegetables were grown on untreated and cyclonic ashes treated plots. Comparison of Cd contents in edible parts of these plants at harvest time (after normal washing) showed strong (factor 2 to factor 4) reductions of total Cd content. During the second year, the Cd guideline value (0.2 mg Cd kg^{-1} fresh weight) for these Cd - accumulating species (spinach, celery and lettuce) were reached in almost all cases, indicating a progressive amelioration of the immobilization of Cd. Durability of metal immobilization in these garden soils by cyclonic ashes was also tested in a simulation experiment.

3. Conclusions

It can be concluded that both field experiments and the simulation experiments suggest a long-term immobilization of the metals resulting in a strongly decreased bioavailability and reduced spread of the contaminants to the surroundings.
Mechanisms of immobilization will be discussed (Mench et al. 1998)

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PROJECT MANAGEMENT AND REMEDIATION DESIGN FOR A METALLURGICAL AND COKING PLANT IN LÜBECK

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Introduction

The site is located in northern Germany, 60 km northeast of Hamburg and has a size of 200 acres (80 ha). Coke production, iron and copper smelting, and the treatment of by-products of those operations, led to significant on site environmental issues. Analysis showed that the site will have limited use due to the more than 1.5 million m³ (1.9 mio. cubic yards) of contaminated residues and waste material that cannot be completely treated or disposed of within a realistic budget. As a result, the remediation goal is long-term prevention of contaminant migration from the site.

1. Site history

After more than 80 years of industrial activities, bankruptcy proceedings in August 1990 ended the site production history. The site was first developed in 1907 with blast furnaces, a coking plant, and ammonium and tar production plants. The site harbor and a connection to the public railway line were established. In 1930, the coking plant produced the entire gas supply for the city of Lübeck. Other production units (benzene plant, tar distillation, portland cement factory, copper and zinc smelter) later followed.

For more than 80 years, illuminating-gas, coke, benzene, ammonium, naphthalene, pitch, road tar, pig iron, iron portland, metal powder, concrete products, zinc/copper products and other by-products were produced. For decades. contaminated residuals like slag, ashes, and sludge were dumped on site and in the adjacent river with no control of leachates or other potential emissions. Reports of fish dying in the Trave River began once on site operations started in 1907. Since then, these issues have caused battle between local fisherman, environmental groups, and the government.

2. Description of the site

The subsurface of the site consists of heterogeneous-fill between 0.3- and 28-m-thick. Slag and construction waste was also used to fill a bay in the Trave River. As a result, the river bank was artificially moved 80 m to the southeast.

Throughout most of the site, the first aquifer, consisting of sand (14-m-thick), is immediately below the fill and is contaminated with organic and inorganic substances. The layer between the first and the second aquifer, used for drinking water supply, is an acquifer consisting of 40-m of glacial clay to prevent contaminants of the first aquifer to migrate into the second aquifer. The groundwater flow of the first aquifer flows southwesterly toward the Trave River. The groundwater flow of the second aquifer flows in the opposite direction, northeasterly, because of a drawdown cone for drinking water catchment in this area.

3. Determination of future use of the site and development of a land use plan

Based on heavy contamination found on site, it was not considered possible to completely remove all contamination within a realistic budget. First, cost estimates showed that if all contaminated material is excavated and disposed of or treated, remediation and redevelopment cost is approximately U.S.$890 million.

It was decided that a certain level of on site contamination would remain, resulting in limited use of the area. Therefore, the site was excluded from residential or agricultural use. Although an industrial/commercial zoning was assigned to the site, nearby residents' needs were considered. It was decided that only those companies whose activities do not impact the neighboring residents' quality of life may operate on site.

Approximately 60 percent of the site will be redeveloped into a commercial/industrial area. The remaining 40 percent will be developed into green spaces.

4. Remediation strategy

The following remediation strategy was developed:

- **Immediate measures to avoid further contamination**

Sources of contamination were located and measures were taken to stop further contamination from leaking pipes, tanks, etc.
A sheet pile wall used to collect contaminated groundwater of the first aquifer and treat the water in a treatment plant, is located downgradient of the coking plant area. The water is treated in a multi-phase separator and filtered with activated carbon before it drains into the river.
Highly contaminated areas with potential for toxic emissions were covered and secured with fences.

- **Protection of drinking water aquifer**

Production wells that allowed hydraulic connection between aquifers 1 and 2 were refilled to avoid contamination of the 2. aquifer (used for drinking water supply).

- **Demolition of contaminated buildings and plants**

All potentially contaminated building material was analysed. All asbestos was removed from buildings, pipes, and parts of the plant.
Where possible, materials were cleaned (*i.e.*, pipes) and then recycled. Contaminated construction material was crushed, mixed with soil, and disposed of at the on site landfill to shape the surface for the planned surface sealing system. Former underground utilities were removed or destroyed and the cavities filled.

5. Project cost

Cost for remediation (investigation measures, remediation planning, and design)	U.S$85 million
Cost for redevelopment (road construction, underground utilities, etc.)	U.S.$8 million
Proceeds from property sales (Price per m^2 U.S.$55)	U.S.$19 million
To be paid by city and State Government	**U.S.$74 Million**

MINIMISING THE BIOAVAILABILITY OF METAL CONTAMINANTS USING NATURALLY OCCURRING MINERALS

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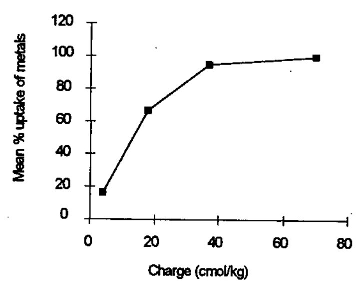
1. Introduction

Aluminosilicate clay minerals are ubiquitous in soils and altered rocks, hence are inexpensive. They are non-toxic. They have high surface areas and commonly carry a permanent negative charge, leading to a cation exchange capacity. Some swell in water. As a result of these properties, they are useful for minimising the bioavailability of metal contaminants.

Results are presented of experiments showing: (a) their capacities to adsorb different cationic metal contaminants, (b) the relative strengths of binding of these cations against desorption by both water and salts, (c) a simple means of enhancing the binding of the cations to the minerals, (d) modifications designed to enable the uptake of anionic contaminants by aluminosilicates, and (d) the effectiveness of a particular bentonite for minimising the permeability of water without swelling.

Fig. 1. % uptake of Cr(III)+Ni+Cd+Pb from 100ml of 1000mg kg^{-1} by 15g of clays in relation to their charge

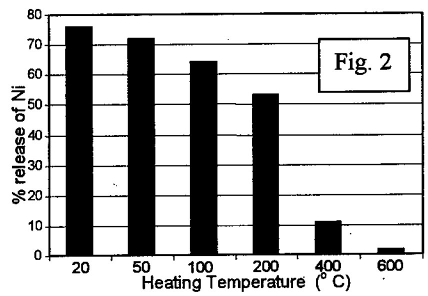


2. Experiments

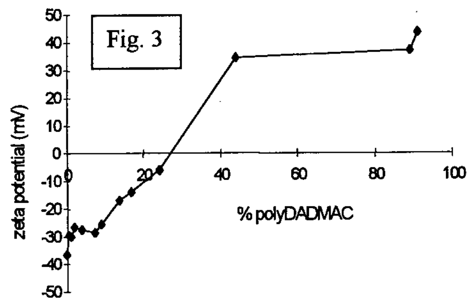
(a) <u>Adsorption of cations by clays</u>. The uptake of Cr, Ni, Cd and Pb by a kaolinite K, an illite I, a palygorskite P and a smectite S was related to the charge on the clay mineral. Figure1 shows the mean % uptake of all of the metals by 15g of clay from 100ml of solutions 1000mg kg-1 in salts of each cation increased with the charge (cation exchange capacity) of the clay. Their relative abilities to remove metals were: S>P>I>K. The relative ease of uptake of the metals was usually Pb>Cd>Ni>Cr, but the order varied with clay type.

(b) <u>Relative strengths of binding of metals.</u>
Samples of a commercial Wyoming bentonite saturated with each of Cr , Ni, Cd and Pb were leached with water and with NaCl or $NaNO_3$. ≤3% of each of the metal cations were removed by water. 0.1M Na salts displaced 20% (Cr) to 76% (Ni) of metals; 1M salts displaced 63% (Pb) to 100% (Cr). Both 0.1M and 1M NaCl displaced 68% of Cd. The ease of displacement with 1M salts was in inverse order of the ionic radii.

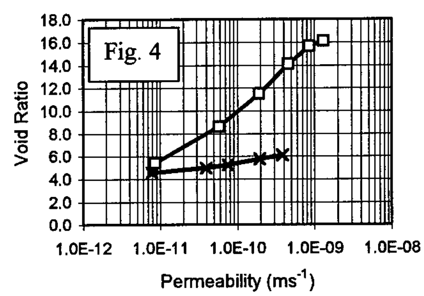
(c) Enhancement of binding of metals.
Heating clays enhanced binding of metals. Figure 2 shows an incremental decrease in cation released by 0.1M NaCl in the case of Ni. There were similar effects for the other metals. Heating also enhanced binding against displacement by 1M Na salts. Heating at 600°C reduced release of all metals by both 0.1M and 1M Na salts to ≤ 10%.



(d) Modification for anion uptake.
Addition of cationic polyelectrolytes, such as polyDADMAC, commonly used in water treatment, decreased the negative charge on smectites, such as Wyoming bentonite, as shown in Figure 3 . The charge is reversed with sufficient polymer, so that the material becomes positively charged and useful as an anion exchanger, e.g. to bind As and Cr(VI).



(e) A non-cracking clay for barriers.
Figure 4 shows permeability in relation to void ratio, which reflects water content, as a Wyoming bentonite W (squares, upper plot) and Arumpo bentonite A (asterisks, lower plot), from Australia, were subjected to increased stress (up to 173 kPa). Each attained similarly low permeabilities. The Figure also shows that, while W can swell (under low stress), A does not swell. Arumpo bentonite has very fine particles, hence a low permeability with little swelling, so little cracking would occur on drying.



3. Conclusions

In order to minimise their bioavailability, metals may be bound to aluminosilicate clays. In water, sorbed metals are hardly lost but are displaced by salts to an extent that apparently depends on their ionic radii. They can be held more strongly by pre-heated clays. The clays can be modified to enable the uptake of metallic anions. They may also form barriers which do not swell (or crack), in order to confine metals.

Oxides and oxyhydroxides of Fe and Al with variable charges may also be used for the sorption of metals. Like aluminosilicate clays, these may either be obtained from concentrated deposits for addition to contaminated sites or else used *in situ*.

STRATEGIES FOR MODELLING AND MANAGING DIFFUSE METAL CONTAMINATION OF RURAL SOILS

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1. Introduction

During the past two decades, there has been increasing concern regarding issues of soil contamination with a considerable range of potentially toxic inorganic and organic pollutants. Investigations have tended to concentrate on those sites that are very heavily contaminated, usually as a result of some form of industrial activity. However, not all soil contamination is localised, or can be attributed to single point sources. In the case of heavy metals, increasing attention is being directed towards more diffuse forms of soil contamination. This type of contamination, although often at considerably lower concentrations than that associated with point sources, can affect very large areas of rural soils. For example, large areas of soils in both Australia and New Zealand have received significant amounts of cadmium (Cd) as an impurity of phosphatic fertilisers. Similarly, the agricultural use of pesticides has resulted in the diffuse accumulation of lead (Pb) and arsenic (As) in soils. Rural soils can also receive metals in manures, irrigation waters, sewage sludge and as atmospheric deposition. Accumulation of metals in soils above certain concentrations may adversely affect the growth of crops and grazing animals, result in increased metal concentrations in plant and animal products, and thus pose risks to human health. There is also increasing evidence that soil biological and biochemical processes may be adversely affected by elevated metal concentrations in the soil. According to Moolenaar et al. (1997), prevention of heavy metal accumulation is one of the prerequisites for sustainable agricultural production. Thus ensuring that "the productivity of the soil and quality of produce is protected but at the same time the ecological functioning of the soil is not damaged, nor should metal emissions from the soil affect other environmental compartments".

2. Management of Diffuse Metal Contamination

There are several possible approaches to the management of diffuse metal contamination. In recent years, considerable attention has been directed towards determining "safe levels" for burdening the soil environment with metals. This has been done essentially to aid the development of strategies for the control of future metal additions, and to assess the risk associated with existing levels of metal contamination. However, contrasting methods have been used in setting metal limits in soils, resulting in widely different numerical limits being set for the same metal (McGrath et al., 1994). Strategies for the management of soil metal contamination should be based firmly on our existing knowledge of metal reactions in soils. Current soil metal loading guidelines or regulations are based almost exclusively on total soil metal concentrations. However, biological responses or metal mobility in soils are likely to be much more predictable on the basis of metal lability or activity rather than on the basis of total metal loading or concentration. McBride et al. (1997) have recently developed a semiempirical equation which relates the metal activity or concentration of soil solutions to the soil's pH, organic matter content and total metal content. Equations of this type may be useful in developing metal loading limits in soils, assuming that there is information available on metal

solubilities or activities in soil solutions that correspond to toxicity thresholds for soil organisms or crops. In general, all the main methods used currently to set metal limits are relatively ineffective in predicting long-term trends in metal bioavailability and mobility. We suggest that the dynamic metal balance model as described by Moolenaar et al. (1997) warrants closer examination for assessing the sustainability of metal additions to agro-ecosystems.

Managing existing metal contamination requires somewhat different strategies from managing future metal inputs to the soil. In some cases, natural attenuation processes may reduce the effects of contamination. Some research indicates that metal solubility and bioavailability, and the ability of metals to desorb back into solution from soil surfaces decreases with time. There is also some evidence that, in the long-term (several decades), metal leaching losses from contaminated soils may be appreciable. Pro-active management of metal contamination, could include alteration of soil properties known to have a large effect on metal solubility, for example, soil pH or soil organic matter content. Addition of other materials, such as iron and manganese oxides, to "chemically stabilise" metals in the soil may also be a possibility. However, whether short-term reductions in metal solubility observed in laboratory studies occur and, more importantly, persist in the field remains to be seen. The same degree of uncertainty could also apply to the use of phytoextraction for reducing metal contamination. Whether this technique is feasible or appropriate in dealing with relatively low levels of diffuse metal contamination, for example, Cd accumulated as a result of phosphate fertiliser applications, remains to be seen. Another strategy for managing diffuse metal contamination that definitely does have a great deal of potential is crop and cultivar selection. Different crops and even different cultivars of some crops show substantial differences in uptake of metals from the soil, and in their ability to cope with metal contamination. Selecting crops with low uptake of metals will reduce the risk of producing food products with metal concentrations above maximum permitted concentrations.

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UTILITY, HUMAN AND ECOSYSTEM IMPACT, AND REMEDIAL TECHNOLOGIES FOR LEAD IN THE ENVIRONMENT

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1 Introduction

Widespread use of lead common before 1980 caused recognition of the potential human and ecosystem impacts from dispersed lead, which significantly reduced use of this metal in recent decades. Further reductions in Pb use and dispersal are sought, as are methods to characterize risks from, and to remediate the risk of Pb in historically contaminated soils. Demand for lead and associated metals has assured continuous mining and smelting. The industrial utility of Pb resulted in widespread use and dispersal of this metal up until the last few decades when recognition of negative health effects encouraged use of less toxic substitutes. Lead was used in the manufacture of industrial, agricultural, and domestic products: $PbAsO_4$, as a pesticide in orchards; Pb metal, in bullets and batteries. Domestic use also caused exposures: Pb pipes that supplied water; Pb in solder (27-97% Pb) for food cans; on walls in Pb-paint (anti corrosive and good covering properties in addition to use in pigments); and as an anti-knock additive to gasoline. Exposures from automotive emissions and soldered cans have been remarkably reduced in the US since 1980 by regulatory requirements.

2. Nature of Lead Poisoning

The most sensitive human health effect of excessive absorbed Pb is a neurophysiological impairment, which occurs at far lower blood Pb than required to interfere with heme biosynthesis. While acute Pb poisoning was a concern for adults as well as for children earlier this century, chronic Pb poisoning of children is now the primary focus. Within the US, mean child blood levels have declined from over 20 $\mu g\ dL^{-1}$ in the 1960s, to less than 10 $\mu g\ dL^{-1}$ for 90% of preschoolers tested in 1994, but at the same time, blood Pb concentration associated with negative neurological development has also been reduced, from 25 to 10 $\mu g\ dL^{-1}$. The majority of impacted children are clustered in urban areas where, in addition to paint, soil and dust have been identified as significant sources of Pb.

3. Ecosystem Impacts

Ecosystem impacts of Pb contamination are often difficult to isolate as Pb is generally found in combination with elevated concentrations of Zn and Cd. For animals that directly ingest Pb contaminated soils and sediments, elevated Pb concentrations in various tissues as well as acute Pb toxicity have been reported. Generally, a combination of factors interact to destroy a smelter or mining impacted ecosystem. Extensive research on the ecosystem impacts of Zn/Pb smelting showed that soil acidity coupled with elevated soil Zn resulted in phytotoxicity. Loss of vegetative cover caused erosion of the surface soil with loss of phosphate. The remaining soil did not have functional microbial populations. Macro-organisms and the small mammals that prey on them (likely candidates for Pb toxicity) were absent from the soil. For these types of systems, it is most

likely a combination of factors that resulted in the death of the ecosystem. While Pb toxicity was a component, Zn toxicity often needs to be addressed before soil functionality can be restored.

4. Remediation of Lead Contaminated Soils

Two main objectives of remediation of Pb contaminated soils are 1) ecosystem restoration and 2) protecting children's health. Both require defining appropriate indices to measure the success and persistence of remediation. Remediation to protect children has concentrated on risk from ingested soil and dust; understanding is needed regarding the dissolution and absorption of Pb minerals in soils in the human gastric tract. Recognition that altering the mineral form of Pb to highly insoluble, stable forms may result in reduced bioaccessibility supports *in situ* Pb-stabilization; formation of chloropyromorphite through phosphate addition to Pb contaminated soils has been shown in laboratory studies. Field trials are currently underway to test the effectiveness and persistence of soil treatments. Animal feeding studies have traditionally been used as a surrogate to determine a reduction in risk to children. Development of simple chemical *in vitro* tests to replace feeding trials is a major goal of present research. Strong corrections of *in vitro* tests with bioavailability have been reported.

The immediate goal of ecosystem restoration technologies for smelter killed ecosystems is the reestablishment of vegetative cover. Making such soils calcareous and rich in phosphate have supported revegetation where tested; added Fe and Mn oxides have helped in some studies. Our current research has focused on the use of a range of residuals, including municipal biosolids and coal fly ash, to apply phosphate, iron oxides and alkalinity to eliminate phytotoxicity. Specific adsorption of metals, potentially leading to precipitation or occlusion is thought to be the mechanism through which the toxicity of soil Zn, Cd, and Pb is reduced by these amendments. While research on ecosystem restoration includes concern with reducing Pb bioaccessibility, the primary goal is reestablishment of a self-sustaining ecosystem which involves limiting the bioaccessibility of a range of metals as well as restoring a nutrient balance that may have been disturbed by metal contamination. The range of organisms and processes involved in a functional ecosystem also suggest that a single variable such as *in vitro* bioaccessibility of Pb is not sufficient to evaluate the success of a remediation technology. Both areas of research will require some type of quantitative measure to demonstrate that the mineralogical form of Pb (as well as other metals of concern) has been altered before these alternative remediation technologies can achieve public and regulatory acceptance. Recent advances in X-ray adsorption spectroscopy show potential for providing such evidence.

Table 1. Reduction of lead bioavailability as a result of in situ soil amendments. Soils were fed to weanling rats. Values reported are for bone Pb.

	Unamended Soil	1% P as H_3PO_4	2.5% Fe + 0.32% P as TSP
Percent Reduction	---	62	45

ASSESSING THE BIOAVAILABILITY OF TRACE ELEMENTS IN CONTAMINATED LAND USING BIOLOGICAL METHODS

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1. Introduction

Human activities have artificially increased trace metal concentrations in many locations. While some trace metals are essential for plant growth and soil biota, oligo-elements having a known biological function can be toxic at high concentrations or when in a bioavailable form. If trace elements, become mobile, they can pose risk to water quality, plants and the food chain, the ecosystems and man. To ensure the sustainable development of land resources, contaminated land must be controlled or remediated for future uses. The management of contaminated land must be driven by a risk based approach for reaching an objective of « fitness for use » taking into account technical and socio-economical constraints but also human health, the integrity and quality of ecosystems (Caracas, 1998).

2. The concept of bioavailability

The concept of bioavailability is fundamental in the process of contaminated land risk assessment. Bioavailability can be defined for a contaminant as its capacity to be transferred from natural media into living organisms (Juste, 1989)). Consequently, it is one of the major factor's of concentration-effect relationships in terrestrial ecosystems. In the risk assessment chain from contaminated sources to identified targets, the mobility of contaminants in the environment and their bioavailability for living systems are key indicators of potential exposure and impact of toxic compounds.

The aqueous soil solution is the main vector of mobile chemical species from stationary source phases of trace elements to living organisms. But transfers within the stationary phases and through the soil gas phase as volatile compounds (e.g. mercury) occur. Consequently, the bioavailability not only depends on soil-water thermodynamical equilibria but also on potential pathways, mass-transfer limitations and associated structure effects. In addition, the passive or specifically active translocation of trace elements into living systems depends on individuals, populations, communities and their activity which is influenced by other external factors. A direct translocation from other phases than the soil aqueous solution is possible. The complexity of these phenomena, and their multiple parameters, limit our capacity to understand and predict all processes involved in the concept of bioavailability.

3. Methods for assessing the bioavailability of trace elements

The use of different reactants in a sequential leaching or extraction procedure provides interesting results on stocks of elements and their potential mobility and bioavailability (Juste, 1989). Commonly used reactants innclude water, salt solutions, organic acids, chelating agents or mineral strong acids. However, the best solution for assessing bioavailability remains within biological approaches (Mench, 1993)). An understanding of biological processes can enhance our capacity to assess ecosystems and human health risks and to develop biological processes for the remediation of contaminated sites.

4. Biological methods and their implications to the remediation of contaminated land
Biological approaches are based on trace element concentrations and the symptoms of their effects on living systems. These two variables can be related through dose-response relationships. This type of study has the following implications:

First, the impact of trace metals on living organisms in contaminated land especially in plants and invertebrates (Baker and Brooks, 1989) may enable the derivation of biological indicators of contamination which can be used for developing phytoremediation techniques for contaminated soils including phytostabilisation and phytoextraction processes. Second, the study of acute, chronic and genetic symptoms from the biomolecular level to the population level when living organisms are exposed to contaminated land materials or their leachates (Tarradellas et. al., 1997). These studies provide an understanding of dose-response relationships, and development of biological testing methods for the assessment of contaminated land.

Third, the influence of biological systems on the mobility of trace elements especially micro-organisms (Berthelin, 1989) nables development of bioremediation strategies involving the capacity of micro-organisms to modify the nature of trace elements. It can also be used to either mobilise or immobilise trace elements as low or high solubility minerals in the soils or sub-soil systems and to assist an understanding of mycorhizal processes involving symbiotic association between roots and fungi, and their influence on the translocation of trace elements into plant systems.

5. Conclusions
Biological methods seem to be the more convenient way to assess the bioavailability of trace elements, in contaminated land systems. Concentration of trace elements in biological materials, toxic effects induced on living systems and modification of the mobility of trace elements in the environment by biological processes are some important fields of research which can contribute to the assessment of bioavailability and its implications to the risk assessment and the remediation of contaminated land. But the efficiency of phytoremediation techniques, the real significance of contamination bioindicators, the interpretation of bioassays in terms of potential impacts at the ecosystem level and their standardisation at the international level, as well as the optimal process engineering of bioremediation techniques in the field are part of the present scientific and technical challenges.

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REMEDIATION OF AS, PB, CD, AND ZN CONTAMINATED SOILS

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Smelting of non-ferrous metal ores, pesticides, coal combustion, Pb-based paint, and atmospheric deposition have resulted in contamination of many urban soils with As, Pb, Cd, and/or Zn. Chronic exposure to these contaminants can result in human health effects including kidney dysfunction from Cd, skin and internal organ cancers from As, and impaired mental development from Pb. Excessive Zn and Cd in soil causes phytotoxicity. Soil remediation policies and soil cleanup levels are often based on reducing the soil contaminant level to one target value or a site-specific risk-based level. Current policies in many countries would require remediation of thousands of sites and millions of tons of soil. Remediation practices often involve costly excavation and landfilling of contaminated urban soil because it is not possible to achieve cleanup levels in a timely or economical manner.

Considerable research has been directed towards less expensive remediation *in-situ* technologies that remove metal contaminants from soil. Soil washing methods based on using extractants to dissolve contaminants are capable of removal of cationic heavy metals from contaminated soil (Ganguly et al., 1998). Unfortunately, field application of soil washing remediation methods are limited to sandy soils with clay and humic content values <15% (Rulkens et al., 1998). Phytoremediation using Indian mustard (*Brassica juncea*) has been used to reduce Pb levels under field conditions (Blaylock et al.,1997). Metal hyperaccumulator plants have great potential as phytoextractors of Cd, Zn, and Pb from contaminated soil (Chaney et al., 1997; McIntyre and Lewis, 1997). Biomethylation using microbial processes has potential to remediate soil contaminated with arsenic (Basta and Rodriguez, 1997).

In-situ immobilization of metal contaminants is an alternate remediation approach where the metals are not removed from contaminated soil. In this approach, soil treatments and/or additives are used to decrease the solubility and bioavailability of metal contaminants. Phosphorus-based treatments have been very successful in decreasing solubility of Pb, Zn, and Cd (Lambert et al., 1997; McGowen and Basta, 1998; Chlopecka and Adriano, 1997; Zhang and Ryan, 1998).

Most of the newer remediation technologies are less expensive than currently-used methods that rely on excavation and transportation of contaminated soils. However, changes in public policies and risk assessment methods are necessary before many new technologies can be implemented for site cleanup. Phytoremediation and bioremediation may require long periods of time to achieve cleanup goals. In-situ immobilization will require use of new testing methods that reflect contaminant bioavailability and risk (Pierzynski, 1998).

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SOIL-BASED COUNTERMEASURES FOR AND REMEDIATION OF SOILS CONTAMINATED WITH RADIONUCLIDES

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1. Introduction

Soil contamination with radionuclides has been caused by accidental release of radioactive materials from nuclear facilities, and global fallout from atmospheric nuclear bomb testings. Once radionuclides enter the soil, they can move up to food chains, and therefore pose a long-term radiation hazard to human health.

2. Biogeochemical behaviour of radionuclides in soil-plant systems

The fate and pathways of radionuclides in the environment depend largely on their biogeochemical behaviour in soil-plant systems, which is controlled by many environmental factors. The residence time of radionuclides is the consequence of their biogeochemical behaviour, and often varies greatly. For example, a study in Shepelichi of the Chernobyl 30km zone showed that, the residence time of ^{137}Cs was only about 1 year in AoL layer, while 170 years in AoH layer corresponding to the changes in soil properties along the profile(Wang, 1997). Pathways of radionuclides from soil to plant are mainly *via* root uptake, which is influenced by an array of factors, such as soil condition, fertiliser application and root-microbe interactions.

3. Soil-based countermeasures for radionuclides contaminated soils

Agricultural countermeasures, mainly application of fertilisers, have been practiced to reduce the migration of radionuclides from soils, especially after Chernobyl accident. The inhibitory effect of potassium on the uptake radiocaesium by crops has been unequivocally demonstrated in hydroponic experiments (Zhu, 1998; Table 1). This effect was also demonstrated by soil-based experiments. However, the effectiveness of application of fertilisers containing potassium and/or calcium largely depends on fertility status and physio-chemical properties of the soil of concern.

Table 1: Effect of external potassium supply on the accumulation of radiocaesium in spring wheat grown in hydroponic system (kBq g^{-1} dry weight)

External potassium concentration (mg L^{-1})	2	4	20	40
Leaves	18.87±3.40	3.38±0.72	0.71±0.01	0.69±0.15
Grains	20.60±1.57	5.37±1.87	1.05±0.00	0.95±0.13

Source: Zhu, 1998.

4. Bioremediation

Medium- or high-level contamination of soil with radionuclides can occur in the vicinity of nuclear facilities especially in the event of an accident. Remediation of these contaminated sites

physically has proven to be very expensive. Extraction of radionuclides by plants is emerging as a cost-effective alternative. So far, most research has concentrated on radiocaesium, with plant species from the families of Amaranthaceae, Chenopodiaceae and Brassicaceae demonstrating the greatest radiocaesium extraction capacities (Fig. 1, Broadley and Willey, 1997). Data collected so far indicate that it will take more than 15 years to decontaminate a soil using plants (Zhu and Shaw, 1999). Recent phytoremediation trials (Mudiganti and Willey, 1998) have shown that TFs are greater at lower concentrations of soil radionuclides. This raises the possibility that, the time taken to phytoremediate sites contaminated with radionuclides might not be as long as predicted using fixed percentage removal rates (Fig. 2).

5. Socio-economic aspect of remediation of radionuclide-contaminated sites

Environmental contamination with radionuclides has to some degree reduced the public acceptance of modern nuclear technologies. To allay public scepticism as well as education of society on the use of nuclear energy to abate global warming, beleived to be exacerbated by fossil-based energy utilisation, effective and efficient remediation of contaminated sites should be pursued. Cost-benefit analysis of various remediation options is also discussed in this paper.

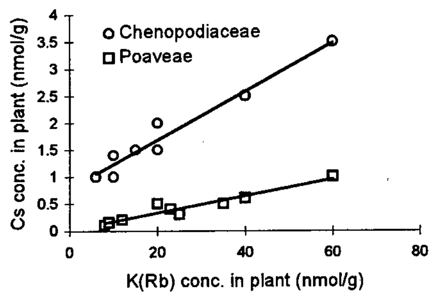

Fig.1: Plants from the family of Chenopodiaceae demonstrate high caesium extraction capacity.

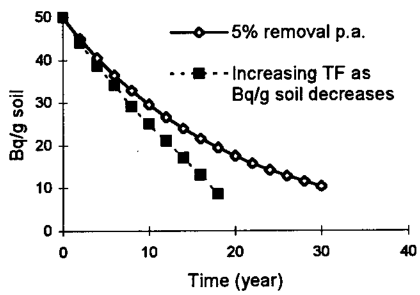

Fig. 2: Increaseing TF as Bq/g soil decreases reduces the duration of phytoremediation.

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USE OF HEAVY METAL RESISTANT BACTERIA IN A BIOREACTOR CONCEPT (BACTERIA METAL SLUDGE REACTOR) TO REMOVE BIOAVAILABLE HEAVY METALS FROM POLLUTED SOILS.

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The presence of heavy metals in the environment, either due to anthropogenic activities or natural causes, has resulted in a continuous selection pressure on the microbial population present at contaminated sites. Depending on the type, toxicity and bioavailability of the heavy metal, bacteria have developed several resistance mechanisms to deal with these metals. Heavy metal resistance can be based on complexation, on modification of the metal to a less toxic form, or on efflux mechanisms that can be combined with post-efflux systems, which prevent heavy metals from re-entering the cell.
Alcaligenes eutrophus strain CH34 is very well adapted to survive in heavy metal polluted environments contains high concentrations of zinc, cadmium, lead and copper. The resistance to zinc, cadmium and also cobalt is encoded by the *czc* operon (Nies *et al.* 1989; Diels *et al.* 1995; van der Lelie *et al.* 1997). The structural *czc* resistance genes form a three-component chemiosmotic efflux system driven by the proton gradient: one metal ion is exported while two protons are simultaneously taken up.
Due to the activity of the *czc* efflux system, a supersaturated zone of metals forms around the cell. Together with high metal concentration, pH increases due to proton transport to the cytoplasm. Carbon dioxide, produced by cellular carbon metabolism, is transformed in this pH gradient into carbonates and bicarbonates, which precipitate with the metal cations that are present in local concentrations higher than their soluble product. This crystallization starts at proteins, induced by some metals, and which function as nucleation sites (Diels *et al.* 1993a and 1993b). This process is supposed to be a post-efflux strategy developed to prevent metal ions re-entering the cell.

Since *A. eutrophus* CH34 was capable of concentrating heavy metals, it was tested whether this strain could be used to remove the bioavailable fraction of heavy metals from zinc and cadmium polluted soils. To do so, cultures of CH34 were mixed with heavy metal polluted soils, and after incubation in the presence of nutrients the mixture was allowed to settle. During settling it was observed that the presence of *A. eutrophus* CH34 resulted in a rapid sedimentation of the soil, while the heavy metal loaded biomass remained in suspension. This soil sedimentation combined with the bioprecipitation process was used to develop a new bioreactor concept to remove heavy metals from polluted soils. This process is referred to as the BMSR (Bacteria Metal Sludge Reactor) concept (Diels, 1997).

A BMSR reactor was constructed that was able to treat 2 tons of soil. After a residence time of 10 hours the soil sludge is settled. The biomass loaded with heavy metals is removed from the water phase. In this way a high concentration of metals is obtained in the biomass. The BMSR reactor gave very good removal of Cd, Zn, and Cu from the polluted soils. Cd could be reduced from 10 – 20 mg /kg to below 2 – 3 mg/kg soil. Pb could also be removed. At present, a reactor able to treat 2 tons of soil in a continuous process is under evaluation. The results of field demonstrations in which the BMSR reactor was used to remove heavy metals from garden soils will be presented.

One important issue is the assessment of the bioavailable fraction of heavy metals before and after treatment of soils in the BMSR reactor. During the full-scale demonstration, this assessment was carried out with phytotoxicity tests and bacterial biosensors for the specific detection of heavy metals. Despite their different mode of action, all heavy metal resistance systems have in common that they are tightly regulated in function of the bioavailable concentration of the heavy metals as detected by the cell. By placing easily detectable reporter systems, such as the *lux* operon of *Vibrio fischeri*, under the control of

regulatory genes of heavy metal resistance operons, biosensors can be constructed that specifically react to the presence of bioavailable heavy metals. This concept was used to construct several heavy metal specific biosensor strains based on *A. eutrophus* CH34, referred to as BIOMET (Corbisier *et al.* 1993, 1994 and 1996).

At present, the following BIOMET strains are commercially available (see Table 1 for their characteristics): AE1433, used for overall toxicity testing and to determine bioavailable zinc and cadmium; AE1239, used for overall toxicity testing and to determine bioavailable copper; AE2448, used for overall toxicity testing and to determine bioavailable lead; and AE2440, used for overall toxicity testing and to determine bioavailable chromium

[only Cr (VI), no induction by Cr (III)].

Strain BIOMET Sensor	Detection of metal	Detection limit (µg/l)	Max S/N ratio	At conc. (mg/l)	Intervention value water (µg/l)	Intervention value soil/sediment (mg/kg d.w.)
AE1239	Cu	64	44	2.8	75	190
AE1433	Zn	164	85	35	800	720
	Cd	11.2	68	22.4	6	12
AE2448	Pb	42	40	62	75	530
AE2440	Cr	25	16	40	30	380

Table 1: Characteristics of the BIOMET tester strains.

Evaluation of bioavailable fractions of heavy metals with the biotests before and after treatment with the BMSR reactor showed a removal of of bioavailable heavy metal fraction. BMSR treatment drastically reduced the metal uptake by plants during the phytotoxicity tests. Also for the BIOMET tests, a decrease in light induction to non-significant levels was observed after BMSR treatment of the soils, indicating a very strong risk reduction. These data prove that the BMSR concept is useful for rapid removal of the bioavailable fraction of heavy metals.

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CURRENT REMEDIATION TECHNOLOGIES IN AUSTRALIA, NEW ZEALAND AND SOUTH PACIFIC

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Contaminated land is an increasingly important environmental, health, economic and planning issue in the Australia, New Zealand and the South Pacific region. A number of initiatives and approaches have been developed for dealing with these issues. As part of this process, Government agencies are placing increasing emphasis on the state of soil environment, recognising the soil's role as a potential repository for pollutants, as a transmitter of undesirable materials to surface and ground waters and as a potential source of contaminants in the food chain.

Increased industrial and agricultural activities, following European settlement of the region, beginning in the early 1800s when regulatory measures were completely absent and environmental awareness low, have led to contamination of the soil environment at two scales. Significant but localised contamination is a feature of industrial or mining activities, whereas agricultural practices and some urban and industrial activities contaminate soils more extensively but at a lower level. The scale of such contaminations varies amongst the regional countries depending on the size of the population and the economy of the country.

The exact number of contaminated sites in the region is not known. It is estimated that there are approximately 60,000 contaminated sites in Australia alone and over 50,000 in New Zealand and the South Pacific Region. Remediation of such contaminated sites could cost more than $A2 billion. Although site remediation is a relatively new concept in the region, the emergence of EPA's throughout Australia and New Zealand has led to extensive expansion of commercial organisations practicing contaminated site remediation in these countries. Remediation, however, is not common in the smaller countries of the South Pacific Region.

Strategies to either manage and/or remediate contaminated sites have developed largely from application of stringent regulatory measures set up to safeguard ecosystem function as well as minimise the potential adverse effects of toxic substances to animal and human health. The processes leading to eventual remediation of contaminated sites involve multiple stages, but generally are initiated with a site sampling and data acquisition stage. In South Australia a relatively new approach is to investigate the current and potential bioavailability of the contaminants and it is only in those sites where contaminants are potentially bioavailable that traditional remediation options are considered. This has emerged particularly where elevated levels of heavy metals are encased in either a vitreous slag or similar *glass like* material following smelting operations - quite common in the Port Adelaide region of South Australia.

Although, limited information is available on the nature of remediation processes in the region, the track record for remediation is not good in Australia. Much expenditure during early remediation has been wasted due to uncertainties with clean-up technologies, inappropriate application of technologies and inexperience or lack of knowledge on the likely benefits and

efficiencies of technologies. Much of the uncertainty has arisen from the lack of appropriate knowledge of the soils and contaminants in the regional environment compared to the country where the technology was developed. Often, contaminated sites contain a mixture of toxic substances and technology developed for the elimination of single contaminant may not be effective at sites containing more than one type of toxic substance. Some examples of such mixed contaminants include gas work sites and mixtures of metal and organic pesticides at former cattle and sheep dip sites. While bioremediation has been found to be effective for a number of organic contaminants, remediation of metal contaminated sites in the region have proved challenging and current strategies include both traditional 'dig and cart' and more rarely, immobilisation as a key management strategy. The emergence in South Australia of a new category of waste - so-called *Intermediate Landfill Cover* - to facilitate acceptance at landfill of low level metal or organic contaminated soils - has resulted in a propensity to dig and cart these materials to situations where their actual bioavailability is likely to increase with time under the influence of mild acetic acid leaching prevalent in landfills. (Table 1). Where the criteria are not met, unscrupulous developers could achieve acceptance using dilution with clean fill - an approach to which the EPA is opposed, but over which it has little control.

Metal	As sulphide in smelter waste (20°C)	As acetate in landfill (20°C)	Increase
Lead	Sparingly soluble	44.3 gm/100ml	> 40 times
Zinc	0.0007 gm/100ml	40.0 gm/100ml	> 57,000 times
Copper	0.00003 gm/100ml	7.3 gm /100ml	> 240,000 times

Table 1 - Comparison of solubility - heavy metal sulphides and acetates.

These poor management approaches have meant that more innovative remediation technologies have lost credibility, and the propensity to apply them has lessened. It is generally accepted that a rational mechanism to address the problem of clean-up of contaminated soil at reasonable cost, needs to be found. Systematic attention to the management and remediation of contaminated land began in Australia in the early 1980s (Barzi et al., 1996). Given that New Zealand also practices guidelines similar to Australia, remediation of contaminated sites could have been established in 1980's as well although no information is available on other regional countries. The available remediation technologies in the region may be grouped into two categories:

- *ex-situ* techniques that require removal of the contaminated soil or ground water for treatment or more typically disposal - either on site or off-site, and in some cases returning the treated soil to the restored site and
- *in-situ* techniques, that attempt to remediate without excavation of contaminated soils.

This paper provides a brief overview of issues of soil contamination within Australia, New Zealand and the South Pacific Region and current assessment and management and remediation strategies that have been developed for addressing these issues.

CURRENT APPROACHES TO MANAGING AND REMEDIATING METAL CONTAMINATED SOILS IN CHINA

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1. Introduction

This paper reviews the current status of derelict land caused by metal mining in China, and provides a summary of current approach to managing and remediating these metal contaminated sites. Examples of reclaiming different metal contaminated areas throughout China are cited. Recommendation is made in order to rectify the present deficiency encountered in reclaiming metal contaminated soils.

Out of the total land of about 9.6 million km^2 in China, only 14.2% is cultivated, 0.5% is under fruit trees, 34.8% is under grazing, 17.2% is under forests, 2.6% is used for industry, communication and urban purposes, and 3.5% is covered by water. Much of the remaining 27.2% is high and barren desert, or under permanent snow cover.

In China, the reserves of tungsten, tin, *stibium*, zinc, titanium, tantalum, thulium rank first, reserves of lead, nickel, mercury, molybdenum and niobium rank second, and the reserve of aluminum ranks fifth in the world. The gross output of 10 main non-ferrous metals increased from 13,000 t in 1949, to 952,400 t in 1978, and 4.25 million t in 1995. The rapid increase in production was largely due to improvement of equipment and technology during end of 1970s and beginning of 1980s.

2. Derelict land caused by mining

There are 477 state owned nonferrous metal mines above country level, with 147 mines directly under the Party Central Committee, and 330 mines operated by country and above country level. Underground mining is the principal mining method, which accounted 70% of the total ore production. In addition, extensive small-scale or artisanal mining plays an important economic role and provides a livelihood for a large number of people in China, and it has been estimated that there are 5 million artisanal miners were working some 280,000 mines in 1994 in China (Industry and Environment, UNEP, 1997). This further aggravates problems of vegetation destruction, hydrological disruption, noise and air pollution, and severe contamination of surface and underground water. The land area disturbed by mining in China is about 2 million ha, and the rate of production of derelict land by mining in China is 20,000 ha $year^{-1}$, and will exceed 33,000 ha $year^{-1}$ by end of this century.

3. Policy for remedation

The Land Reclamation Stipulation issued in November 1988 was put into effect since January 1, 1989, in order to minimize the reduction of land resources and to improve the environmental quality. It clearly stated that: (1) whoever destroy the land is responsible for reclamation, (2) administrative departments of mining companies are in charge of land reclamation, (3) land reclamation should be planned at the very beginning, and should be part of the overall plan of mining companies, (4) mining companies should be responsible for any expenses incurred for land reclamation and any reimbursement made due to land loss, (5) the operation procedures of land reclamation are defined, and (6) the ownership and use right of the rehabilitated land are rationally stipulated.

Land reclamation in China has developed at a slow pace. By the end of 1990, only 2% of the total 2 million ha wasteland had been reclaimed, but of poor quality. The reclamation ratio has increased throughout the years, only 0.7-1% of derelict land was reclaimed at the beginning, about 2% by end of 1980, and 6.67% at the beginning of 1990, and reached 13.33% by 1994. Totally 133,300 ha of land destroyed by mining has been brought back to use in 1994, which equals to the sum of area in 1992 and 1993 (National Land Management Bureau, 1995). However, it has been noted that problems related to land reclamation of mined land included insufficient enforcement of environmental law, lack of initiatives of the mining companies, use of inappropriate reclamation techniques, lack of funding for reclamation and overall planning.

4. Current approaches of reclamation

Examples will be cited to investigate the current approach of reclaiming metal (aluminum, copper, lead/zinc, iron, tin) contaminated soils at different mines throughout China. The results indicated that *in-situ* immobilization together with vegetation is the most effective for the reclamation of metal contaminated areas. This will involve the use of proper inorganic and organic amendments and choice of suitable plant species. In addition, continuous monitoring and aftercare is also important for ensuring durability. Our major concern is the use of reclaimed land for agricultural production, which may impose health hazards, due to uptake of potentially toxic metals by crops. Risk assessment is therefore necessary to reveal the amount of toxic metals taken up by crops, which may subsequently enter in the food chain.

5. Recommendations

It is recommended that government officials, mine operators and scientists should cooperate to tackle the problem. Reclamation of mined land should be included as an integral and important part of mining operation, which should be planned at the very beginning. Deadline should be imposed for reclaiming existing derelict land caused by mining activities. Source of funding and its usage should be explicitly stipulated. Clear guidelines should be provided as to the quality and maintenance of the reclaimed land. In addition, the following points related to reclamation techniques should be noted: (1) Land reclamation is a long term process, and revegetation should be effective and the biodiversity of reclaimed land should be maintained, (2) productivity of planted species should achieve a permitted level, and their survival rate should exceed 90%, (3) the reclaimed land should achieve a better standard or at least maintain the same standard as those before mining, (4) toxic mine wastes should be remediated using inert and organic amendments and planted with appropriate plant species, (5) special attention, especially reclamation techniques should be made, if the reclaimed land were used for agricultural production, (6) similar land morphology should be recovered, and if possible original cover soil should be used for reclamation, (7) the imbalance of material flows between reclaimed land and adjacent areas should be minimized, (8) toxic waste materials, including potentially acidified materials should be properly treated, and (9) special attention should be given to minimize disturbance on wildlife communities, including birds, mammals and fish.

CURRENT REMEDIATION TECHNOLOGIES FOR METAL-CONTAMINATED SOILS IN THE UNITED STATES

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1. Introduction

Numerous metal contaminated sites exist in the United States. As of January 1999 there were 1206 sites on the national priorities list (NPL) through the Superfund program. The metals most often found at the sites involving soil contamination and the percentage of the sites affected were Pb (34%), Cr and As (29%), Cd (22%), Zn (21%), Ni (18%), Hg (16%), and Se (7%). Thousands of other sites exist that are not on the NPL for a variety of reasons. Many of these are present on Department of Defense or Department of Energy facilities, or are too small to have warranted regulatory attention.

These metal contaminated sites may present both human health and ecological risks. Human health risk assessments often find definitive evidence of health effects, such as blood Pb concentrations in children exceeding health guidelines, or indirect evidence such as reference doses that have been exceeded for one or more metals. Some epidemiological studies have found higher incidences of disease in contaminated areas compared to control areas. Ecological risks are not as well characterized. The lack of vegetative growth at many sites is direct evidence of ecological effects from the mining activities. This problem can be attributed to poor soil physical conditions, low soil fertility, and phytotoxicities. The areas of exposed soil and mine wastes further exacerbate the problem as erosion moves metal-rich materials into sensitive aquatic environments and increases human exposure through wind-blown dust. Ecological risk assessments for terrestrial environments also show adverse effects on earthworms and other soil decomposers. Ecological risk assessments for aquatic habitats consistently show water and sediment metal concentrations exceeding toxicity benchmarks with observable impacts on aquatic invertebrates and fish communities.

In general, the remediation efforts for a given site begin by addressing acute human health problems followed by chronic human health problems and then possibly by ecological problems. The primary human exposure pathways that are addressed include incidental ingestion of soil and dust, food-chain exposure through home-grown produce, and ingestion of contaminated drinking water. Other pathways may include inhalation of wind-blown dust, dermal exposure to contaminated soil, and exposure to contaminated surface water during recreational activities.

2. Remediation Strategies

Remediation strategies can be divided into two general categories: *ex situ* and *in situ*. Table 1 presents a summary of technologies that are currently being used or are being evaluated. Soil removal is the most common *ex situ* method employed and is used extensively in residential areas with Pb contaminated soils. Use of the remaining *ex situ* methods is generally restricted to small sites with highly contaminated soils because of the obvious problems with dealing with large quantities of soils and the generation of waste products requiring further processing or storage. Phytostabilization is the most common in situ method used, particularly for old mine sites with large areas that require remediation. Encapsulation and attenuation have been used to a limited extent. The use of soil amendments is often combined with phytostabilization. The use of P to reduce Pb bioavailability is being evaluated as an alternative to soil excavation.

Table 1. Remediation strategies currently employed or under development for metal-contaminated sites in the United States.

Method	Comments
Ex situ	
Solidification	Addition of a cementing agent to produce a hardened, nonporous, nonleachable material
Vitrification	Heating to produce a glass-like, nonporous, nonleachable material
Washing	Chelate or acid extraction
Leaching	Pile or batch leaching with chelates or acids
Particle size segregation	Selective removal of fine particles that have the highest metal concentrations
Soil excavation	Soil removal and disposal
In situ	
Solidification	As described above
Vitrification	As described above
Encapsulation	Cover site with impermeable layer
Attenuation	Dilution with uncontaminated material
Electrokinetics	Electric current induces movement of ions to electrodes
Phytostabilization	Promote vegetative growth to immobilize metals
Phytoextraction	Removal of metals by plants
Soil amendments	Reduce bioavailability of metals with P or other soil amendments

3. Case Studies

Several extensive metal contaminated sites will be used as case studies. These include the Tri-State Mining Region; Palmerton, PA; Bunker Hill, ID; Leadville, CO; and Butte, MT. Some of these sites have been on the NPL for over 15 years and the completed remedial activities illustrate a progression in technologies and philosophies toward remediation. Acute human health problems are still being addressed at many sites with little progress on the ecological risk problems.

EVALUATION OF HEAVY METAL CONTAMINATED SOILS AND REMEDIATION OPTIONS AT SELECTED INDUSTRIAL SITES IN SWEDEN

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1. Introduction

Heavy metal contamination in soils has emerged as one of the key environmental issues in Sweden during the recent years. Due to the lack of regulatory legislation in the past, heavy metal concentrations in soils around several old and abandoned industrial sites have increased manifold relative to their background levels. Swedish Environmental Protection Board (Naturv†rdsverket, 1996) has proposed guideline values for heavy metals in the soils, based on their ecotoxicological behaviour (Viktorin et al. 1991) as well as the nature of future land use. Soils at industry-contaminated sites with metal contents of >15 mg/kg As, >1 mg/kg Hg, >100 mg/kg Cu, >120 mg/kg Cr(III), >5 mg/kg Cr(VI), >400 mg/kg Zn, and >80 mg/kg Pb need attention for remediation for the purpose of sensitive land use. The guideline values for the contaminant metals >15-40 mg/kg As, >4-8 mg/kg Hg, >400 mg/kg Cu, >500 mg/kg Cr(III), >15-20 mg/kg Cr(VI), >1400 mg/kg Zn and >300-600 mg/kg Pb, however vary for less sensitive land use depending on the option of groundwater abstraction.

2. Options for remediation

In spite of the awareness of the potential health hazards of heavy metal contaminated soils, most of the soil remediation techniques in recent years have emphasised in situ immobilisation or excavation of the contaminated soils to some 'safe' depositories (i.e. Lundgren, 1995) thereby leaving the contaminants as potential 'chemical time bombs'. The strategies have been almost identical without any specific bias on the nature of contaminant metal species in the soils and the geochemical environment.

Mobilisation of the metals from contaminated soils through acidification is one of the potential methods for chemical remediation (Bhattacharya et al. 1998). Geochemical processes that control solubility and subsequent removal of heavy metals from the contaminated soils during chemical remediation could be attributed to desorption from the surface-reactive soil components, dissolution of the unstable mineral phases (i.e. carbonates and other metal-oxides) and formation of soluble metal-complexes. The effect of pH on metal solubility has long been demonstrated by several investigations through out the world. However such trends may deviate in the presence of organic matter because formation of metal-organic ligand complexes (Bourg & Schindler, 1985).

3. Case studies

A number of industrial contaminated sites have been chemically remediated using batch studies. The sites represent : i) a former ammunition industry at Zakrisdal, Karlstad in Central Sweden, ii) former wood preservation facilities at: a) Konsterud, Kristinehamn Municipality in Central Sweden, b) Fnasbacken, Ljungby Municipality in Southern Sweden, iii) Hugelsta shooting field and waste ammunition dump of Carl-Gustaf Bofors AB at Eskilstuna in South-Central Sweden. The remediation process involved separation of fine grained fractions that represents the most

reactive component of the contaminated soils. The fine grained soils predominantly comprise clay minerals as well as poorly crystalline secondary surface-reactive aluminosilicate phases like imogolite (ITM) and amorphous oxides of Fe-, Al- and Mn with pH-dependent surface charge. Such surface-reactive soil components are capable of adsorbing both anionic (i.e. AsO4-, H3AsO30, AsO4-, Cr2O72-, CrO42-) and other heavy metals in cationic forms like Cr3+, Cu2+, Zn2+ and Pb2+. Grain-size dependence on heavy metal concentration has a practical significance for remediation as it significantly minimises the effective volume of contaminated mass.

The former ammunition industry of Bofors Weapon Systems at Zakrisdal (1940-1970) manufactured percussion caps/detonators with extensive use of Hg(II)-fulminate and Pb(N3)2 (lead azide) as the explosive chemicals. Prolonged use of these chemicals, series of test explosions and eventual repositories of the waste materials have resulted in accumulation of heavy metals Pb (42-24329 mg/kg), Zn (20-23695 mg/kg), Cu (6-35128 mg/kg), Cr (4-901 mg/kg) and Hg (16-197 mg/kg) in the soils within the area. The sites of old wood preservation facilities ..t Konsterud and at Fnasbacken had elevated concentrations of As, Cu, Cr and Zn due to the use of soluble preservative chemicals and handling of processed wood products. Metal concentration were significantly higher in the <0.125 mm fractions of the soils with concentration range between 45-1627 mg/kg As, 12-289 mg/kg Cu, 6-233 mg/kg Cr and 9-724 mg/kg Zn. Analyses of the 0-2 mm fraction of the wooden chip (bark material) from Fnasbacken revealed Cu and As contents of 6134 and 66 mg/kg respectively, while the strand sediments contained 1414-5057 mg/kg Cu and 47-55 mg/kg As (Bhattacharya & Jacks, 1995). Composite soil sample around Hugelsta shooting field and the waste ammunition dumps of the ammunition industry of C-G Bofors, Eskilstuna revealed average total Pb concentration of 16344 mg/kg in the 0-1 mm fraction. In general, the heavy metal concentrations were found to be elevated by a factor of 2-100 compared to the guideline values.

Remediation experiments were carried out on the contaminated soil materials using different organic (oxalate) as well as inorganic (chloride) extractants under different pH conditions. The fine grained fractions of the soils containing the bulk of metal contaminants were chosen for these batch experiments in order to establish the effectiveness of chemical remediation. The quantity of the metals extracted varied with the soil characteristics and the concentration and pH of the extraction media. Extraction of fine-grained fractions of the contaminated soils from the wood preservation site at Konsterud revealed removal of ca. 93-99% As, 60-66% Cu, 67-70% Cr and 56-65% Zn. An effective recovery of about 96-98% of the contaminant metals As and Cu were achieved from the contaminated soil materials from Fnasbacken (Bhattacharya & Jacks, 1995). Nearly 72% of the total Pb was leached in a single step of sequential leaching for the composite soil sample from Hugelsta shooting field. Distinct variability of the recovery of the contaminant metals was observed depending on the soil characteristics. The process leachates were regenerated through ion exchange column and the contaminant metal species were later isolated by a suitable eluent. The major target of metal recycling can also be further carried out by electrochemical techniques.

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BIOREMEDIATION OF METAL CONTAMINATED SOILS – STRATEGIES FOR INDIA AND NEIGHBOURING COUNTRIES

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India and its neighboring countries such as Pakistan, Bangladesh, Thailand and Malaysia have witnessed rapid industrial development in the last 2-3 decades. The protection hereto provided to the industry and the emphasis on decentralization of industrial development have resulted in serious environmental pollution problems related to the release of a variety of toxic and hazardous substances. Relatively few of the known hazardous waste management tools such as physico-chemical processes, thermal processes and biotechnological processes are presently in use, especially in countries of the Indian subcontinent.

Among the various toxic and hazardous substances, metals have a unique place. Although some metals are essential for life, they become toxic at higher concentrations and have to be removed from contaminating environments, such as air, water and soils. Metal contamination may be anthropogenic as well as geogenic. For example, disposal of chromium-containing tannery waste, deposition of flyash, phospho-gypsum, iron and steel sludges, manganese and tin fines from ore washing plants are major factors that contribute to metal contamination of soils in India and neighboring countries. While these problems are due to human activity, chromium contamination of soils in areas having chromite deposits (Orissa, India) or presence of arsenic in groundwater and soils (West Bengal, India and Bangladesh) are notorious examples of geogenic metal contamination. It is important to note that soils contaminated with metals often pollute groundwater resources. Therefore, remediation strategies must be developed for these two ecosystems. Unlike organic compounds, metals are not degradable and therefore pose a challenging problem to scientists and technologists working on remediation strategies. Since metals are also a non-renewable, finite natural resource, the challenge is not limited to their removal, but may extend to finding efficient and economic ways of recovery and recycling.

Conventional remedial solutions for contaminated soils (excavation and incineration) and groundwater (pump-and-treat) are slow, expensive, increase inputs to hazardous waste disposal sites, and can increase human exposure to contaminants. Bioremediation–the use of microorganisms to destroy hazardous contaminants or to convert them to harmless forms–is an emerging treatment technology that can in many instances restore contaminated environments more quickly, at lower cost, and at lower human risk than alternative remediation technologies. Bioremediation can operate in either an *in situ* mode where contaminants are treated in place, or in an *ex situ* mode where contaminants are removed from a contaminated zone for treatment (preferably on-site). When soils and groundwater are contaminated with metals, bioremediation alternatives are limited to mobilization or immobilization. The choice of mobilization or immobilization is dependent upon the chemical properties of the metal contaminant, the geochemistry of site soils and hydrogeologic setting of the site.

Microorganisms have evolved several strategies to deal with metals including :

- Precipitation
  - Intracellular accumulation and complexation
  - Cell wall biosorption and bioimmobilization
  - Production of exopolysaccharides
  - Volatilization
  - Efflux systems
  - Valence transformation

Using one or a combination of more than one methods as above, various processes have been developed by researchers for mobilizing or immobilizing metals from contaminated environments. Processes that are of immediate relevance to India and neighboring countries will be described along with appropriate case histories. Some examples are listed below:

- Microbiological processes for the treatment of hexavalent chromium containing soils and waters
- Biotechnological methods for removal of arsenic from soils and groundwater
- Bioleaching of copper from waste ores
- Microbial removal of manganese fines from ore washing plants
- Microbiological studies on tin mining areas
- Use of microorganisms for increasing micronutrient availability in soils
- Microbiological reclamation of alkaline and saline soils
- Development of metal biosorbent granules for removal and recovery of toxic and precious metals
- Bioremediation of metal-cyanides
- Microbiological removal of metals from fly ash
- Use of humic substances in remediation of metal-contaminated environments
- Bacteria associated with zero-valence iron and enhancement of metal precipitation
- Use of constructed wetland systems for treatment of metal contaminated waters
- Deep-planted poplar trees as a vegetative remediation strategy

REHABILITATION OF CONTAMINATED LAND : A REGIONAL EUROPEAN PERSPECTIVE

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The majority of the contaminated sites in Europe are the product of the first phase of the industrial revolution (1800 - 1945) and predate current concerns that relate to environmental quality parameters. As Europe industrialised, contaminated sites that arose from manufacturing became integrated into the general urban community with the result that potential exposure of large population centres became commonplace as workers dwellings became intimately associated with their places of employment. Changes in industrial activity since 1945 have lead to the closure of a wide range of generic processes (eg plants for gas manufacture) as a result of economic factors or changing markets.

The main consequences of industrial repositioning have been the replacement of older, pollution-generating industries with new cleaner technology, reflecting changes in consumer demand and the major changes in legislation relating to industrial discharges and disposal of wastes. Industry has also relocated to areas with skills bases that match changing needs. This has left large areas of derelict land once associated with manufacturing in outer city locations (between the commercial centre of conurbations and the outer regions of domestic housing.) These brownfield sites are now being increasingly targeted as areas to be redeveloped for housing, commerce, light industry and recreation, their redevelopment frequently being an integrated part of a focused urban regeneration plan.

The commercial value of derelict land is such that redevelopment takes priority over rehabilitation. Current land values in Merseyside, UK, an Objective 1 region within the EC, are typically in the order of £1 million/ha for a development site that meets UK government guidelines on soil contamination. This, coupled with financial incentives to redevelop parcels of contaminated land has led to the development and utilisation of remediation technologies that deliver land with acceptable levels of contaminants to enable it to be commercially utilised. As many urban sites are developed for commerce, as opposed to housing or urban greenspace, the target values are less onerous to achieve and there is no requirement to meet criteria associated with multifunctionality. In consequence, technologies that change the structure of the soil are frequently employed (eg soil washing) as well as those that disperse and dilute (soil mixing, removal to landfill).

Using Merseyside as an example, it is possible to examine attitudes to derelict land and rehabilitation, based upon changes in legislation and economic climate. Within the region, there is a history of heavy industry that has created severe soil contamination over a period of approximately 150 years (Pilkington plc 1999). At the forefront of this was the Leblanc process for the manufacture of soda ash. The centre of St Helens was a major location of this process, which generated very large volumes of alkaline As-contaminated waste, known locally as "gallegue". During the latter half of the 19th century, this material was spread over large areas of land that were subsequently re-developed for further industrial use. In some areas, deposits of this waste reach depths of 15 metres, covering areas of several hectares. Arsenic levels are in the

range of 200 - 2000 µg g^{-1} dry wt, significantly exceeding current UK land use guidelines. Rehabilitation of these soils, following the demise of the industries that were subsequently developed on top of the contamination, represents a massive undertaking that would not be covered by subsequent commercial revenue. In addition, the landholder is deemed responsible for the cost of rehabilitation, as previous owners are frequently insolvent or have disappeared. One area of contaminated land, comprising a tip containing >6% As has been redeveloped by a local authority/private sector partnership and a further area is currently being considered. Reclamation at this site will be based on civil engineering solutions, due to size and time constraints.

Metal refining and processing activities in the region have generated significant areas of contaminated land. Industries based on copper and tin refining and processing have flourished and in some cases declined over the last century, leaving a legacy of contaminated land. Approaches to the development of the subsequent contaminated sites illustrate a strong *laissez-faire* attitude prevalent in local authorities in the UK as a whole. The copper refinery and cadmium alloy plant at Prescot was finally demolished in the mid 1980's, leaving a large development site adjacent to the town centre that contained elevated soil copper and cadmium levels (Dickinson et al 1996). This site has not been developed, due to the cost involved in rehabilitation in relation to the potential return on such an investment.

Similar attitudes were evident in relation to the treatment of the site of the final tin smelter within the Liverpool city boundaries. This site ceased production in 1964 and was decommissioned in 1965. The site was contaminated with As (>1%), Sb (5-700 µg g^{-1} dry wt) and Cu. The factory was originally within Liverpool, but local government re-organisation left 50% of the site in one authority and 50% in another. One half of the derelict land was covered with no further treatment and converted into a play area, whilst the remainder was left derelict for 25 years with no incentive to re-develop. This has now been covered and converted into urban greenspace.
Current attitudes to rehabilitation are evident at the site of a former Tar distillery, located north of Liverpool city centre, at a site with considerable commercial potential. Here, 2-3 ha of land heavily contaminated with organic and inorganic pollutants are being remediated to a standard suitable for commercial development. A range of techniques are being employed to reduce levels of organic pollutants (PAH, Phenols), based upon the biodegradation of the organics. Contaminated soil has been amended with synthetic zeolites to immobilise labile metals and the natural hydrocarbon-degrading microflora has been encouraged by this measure and frequent aeration of windrowed soil. There is considerable commercial value in the site, so rapid remediation techniques are employed that reflect the need for speedy and efficient rehabilitation. Commercial pressures are probably the main driver behind the methods of rehabilitation used on contaminated soils in Europe. The above examples from one region illustrate this quite clearly and demonstrate that rapid methods are considered most appropriate at sites with clear commercial potential, whereas non-commercial sites are frequently left untreated or subjected to minimum acceptable standards. Only these latter sites would represent opportunities for plant-based remediation strategies, based upon stabilisation of the status quo, subject to the budgetary constraints that will operate around their maintenance.

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Trace Element Issues in Developing Countries

(Special Symposium 7)

CONTAMINATION OF HUNGARIAN SOILS BY TRACE ELEMENTS

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1. Introduction

Hungary is situated in Central-Eastern Europe, geographically in the Carpathian basin, socially and economically between the more industrialized countries of Western Europe and the developing nations of Eastern Europe. The geographical circumstances give many possibilities and raise many problems. More than 85% of the whole territory is used in agricultural production or covered with forests, but the soil map shows a mosaic-like pattern of great variability, and about 50% of the whole land area is subjected to factors limiting soil fertility. The climate is mainly continental, with somewhat less rainfall than needed for the agriculture. The majority of surface waters (96%) arrive from the neighbouring countries, but much of the incoming water is already contaminated with inorganic and organic pollutants.

2. Trace elements in agricultural soils, background values

Data on natural trace element concentrations of the soils are available from earlier studies on the microelement- and potentially toxic element contents of agricultural fields. In a FAO study the micronutrient contents of Hungarian soils were at the international level, with B slightly on the high side, but Cu, Fe, Mn, Mo, and Zn on the low side (SILLANPÄÄ, 1982). In the 80's, in a representative survey including 6000 sampling sites, the plant-available B, Cd, Cr, Co, Cu, Hg, Mo, Ni, Pb, Se, and Zn contents were mostly on the low side of the range of natural occurence (cit. in CSATHÓ, 1994). The main sources of trace element contamination in agricultural fields are dry and wet deposition, application of fertilizers, certain pesticides, and sewage sludges. Trace element content and application practice of such materials has been regulated even more strictly than in some EU countries. As a result, the agricultural soils are still relatively clean of trace metal contaminants. However, in later studies in some areas higher Cd and Pb contents were found, due to aerial deposition from traffic (KÁDÁR, 1995).

3. Trace elements in urban and industrial sites

The trace element contents in the urban soils are generally higher than in the uncontaminated agricultural soils. Increases in the Cd, Cu, Na, Pb, S, and Zn contents were in the range of an order of magnitude (KÁDÁR, 1995). The studies showed significant differences within the same municipality, reflecting the density of the population, the intensity of the traffic, and the extent of industrial activities in the different parts of the town. Studies show significant increases in soil's Pb content in the vicinity of highways.

4. Case studies of industrial contamination

Former old-fashioned industrial processes (mostly of the mining, metal-processing, electro-plating and leather industries), the deposition of hazardous waste materials containing very high amounts of toxic elements, and especially the improper handling of such wastes, caused in some instances

serious soil contaminations in the surroundings of the factories or the deposits. Arsenic, Cd, Cr, Cu, Ni, Pb, and Zn rates exceeded several times the permitted soil levels, sometimes concentrations of several thousand mg/kg soil were found (cit. in CSATHÓ, 1994). Detailed data are available in most cases about the extent and location of such contaminations, and measures were implemented to stop the improper activities, to avoid consumption of possibly contaminated food from the sites and finally, to remediate the sites.

5. Assessment and remediation of contaminated soils

In the past years an Information and Monitoring System for Soil Protection was organized, using 1200 sampling points to follow changes in the status of Hungarian agricultural soils. Nearly 200 of these points are located at "problematic" sites, yielding information about possible diffuse contamination (MOA, 1995). Point-source pollutions present a different task. Guidelines for assessment and qualification of such soils and a new set of limit values for inorganic and organic pollutants in soils and water resources are in the process of elaboration, based on the threshold levels established in other countries and in the EU. Contamination cases are registered at the regional Environmental Protection Agencies, which help also to establish the measures for damage control. On the majority of such sites some remediation process was already done or is in progress. The actual work is done mostly by various private companies. On sites highly contaminated with metals it is often necessary to change the soil.

6. Conclusions

On the verge of joining the EU, Hungary has to take advantage of her uncontaminated agricultural soils and must protect them; at the same time implementing up-to-date industrial technologies to avoid further pollution and current environmental technologies to remediate already contaminated areas.

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SOIL CONTAMINATION AND TRACE ELEMENTS ISSUES IN TAIWAN

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1. Introduction

Taiwan is located in the subtropical and tropical regions with high precipitation (>2500 mm/yr.) and high temperature (>22°C). One fourth of the total area is for rice and upland crop production. Taiwan is a very urbanized country. There are more than 21 million people living on this island where only 30% of the land is less than 100 m above sea level. More than 70% of the total population live in urban areas. The objectives of this paper are to describe (1) the nature and concentration of contaminants in rural and urban soils, (2) current methods for assessing the contamination sites of rural and urban soils, and (3) issues of soil contaminants in Taiwan.

2. Industrial development and solid waste production

There are about 100, 000 registered industrial businesses in this island and about 20, 000 of these businesses produce wastewater. Currently the government agency has established a very detailed information system for regarding the wastewater discharge. Most of the factories are located in the 88 industrial parks administrated by the Bureau of Industrial Development. Thirty industrial parks offer central wastewater collection and treatment systems to treat various industrial wastewater within parks. The methods to distinguish hazardous wastes from the general industrial waste follow the system of USEPA. It is estimated that 30, 000, 000 tons of industrial wastes are generated each year and that classified hazardous wastes make up about 2, 900, 000 tons (9.7% of total).

3. Sources of soil contamination in Taiwan

The most serious sources of soil contamination recognized by the government are (1) hazardous waste heavy metals, (2) hazardous organic waste materials, and (3) corrosive metal waste materials. Contaminated sites always result from wastewater disposals with very high concentration of heavy metals. 80% of soil pollution has been mainly contributed by wastewater discharged from chemical plants and metal surface treatment plants in the industrial parks (CHEN, 1992). Many small works or plants have been located in the urban sites of Taiwan since 1980s. The rural soils surrounding the cities have been contaminated by discharged wastewater.

4. Potential heavy metal contaminated sites in Taiwan

About 800 ha of rural soils were regarded as potential heavy metals contaminated sites. The concentrations of trace elements extraction by 0.1M HCl are higher than the monitoring values proposed by EPA-Taiwan (mg/kg dried soils): Cu 100, Cd 10, Cr 16, Ni 100, Pb 120, and Zn 80. The major heavy metals in these potential contaminated rural soils are Cd, Cu, Cr, Ni, and Zn. Two serious Cd- and Pb-contaminated sites have been announced by the EPA-Taiwan in 1990s, caused by the waste water discharged from chemical plants which produce plastic stabilizing materials (CHEN, 1992; LEE and CHEN, 1994). The mean concentration (2.5 mg/kg) of Cd in brown rice grown in these contaminated soils are higher than the maximum permissible Cd concentration for diet rice (0.5 mg/kg) proposed by the Department of Health (DOH) of government. Potential contaminated urban sites in Taiwan were located in Taichung and Chunghwa city, central Taiwan, especially contaminated by Cd, Cr, Cu, Ni, Pb and Zn.

5. Critical concentration used to assess the heavy metal polluted sites and monitoring sites in the developed countries

Various approaches for assessing the contaminated soils are used internationally, especially in the developed countries (ICRCL, 1987; USEPA, 1989; ALLOWAY, 1990; TILLER, 1992). Many national governments, states, or local authorities without their own formal guidelines or regulations, have used the Dutch Standard to support their decision on assessing contaminated sites or monitoring sites. The guidelines of trace elements in soils were proposed including a target value (normal or natural level), limit value (A value for top of acceptable range); intervention values (B values for further investigation or regulation, and C values for cleaning up).

6. Taiwan approaches

The guideline for assessing heavy metal polluted sites and monitoring sites in Taiwan are primarily based on basic soil properties in Taiwan and the effects of heavy metals on (i) water quality, (ii) activity of soil microorganisms, (iii) human health, and (iv) plant productivity and crop quality. Final guideline values for evaluating the soil quality were proposed by the working group of EPA-Taiwan established in 1990. The top values of the background concentration (A value), the proposed monitoring values (B value), and the proposed cleanup values in the contaminated sites (C value) of heavy metals were developed in Taiwan in 1996. These guidelines will be revised in 1999. The contents of guidelines include phyto-available content (extracted with 0.1M HCl) and total contents.

7. Priority soil contaminants issues in Taiwan

Hazardous heavy metals are recognized as first priority contaminants by EPA-Taiwan on potential contaminated sites in Taiwan, especially As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn. Recommended processes for the assessment and management of contaminated sites have been developed. Remediation techniques have been applied to field scale to clean up two Cd- and Pb-contaminated sites and one organic pollutants site in 1998. The other priority contaminants recognized by EPA-Taiwan will be hazardous organic waste materials including medical chemical compounds, oil chemical productions, pesticide manufacture, and formulations. Guideline values of these organic and inorganic contaminants in soils will be also revised by EPA-Taiwan in 1999.

8. Conclusions

The sources of contaminants in rural and urban soils in Taiwan are mainly from heavy metal-containing wastewater, organic waste, and corrosive metal waste. Some sites have been announced as Cd- and Pb-contaminated sites based on the guideline values of soil quality and human health. Some urban soils in central Taiwan are also contaminated by organic pollutants. The approach processes of reference background levels, monitoring levels, and clean up levels of soil pollutants have been proposed. The assessment for potential contaminated sites is also established in Taiwan. Some remediation techniques have been applied to a field scale to clean up the heavy metal contaminated sites and organic pollutants site in 1998.

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ENVIRONMENTAL AND AGRICULTURAL ASPECTS OF TRACE METALS IN SOILS OF POLAND

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1. Introduction

Surficial quaternary sediments covering the major part of the territory of Poland (located in Europe roughly between the longitude 14° - 24° and latitude 49° - 55°, mainly within the region of marine cool temperate climate) are composed of glacial till and outwash with predomination of sand deposits. Soils developed under these conditions belong, in majority, to Orthic Luvisols, Podzols, and Eutric and Dystric Cambisols (FAO/UNESCO soil groups). Thus, pristine contents of trace metals of soils are rather low, and in some cases are deficient for crop plants.

2. Background contents and metal pollution of soils

Soils of Poland, as in other European countries, have been exposed for a long period of time to relatively large emissions of trace metal pollutants. However, light soil texture, low pH values, and the predomination of leaching processes have not stimulated a great accumulation of metals in surface soils. The contemporary average background levels of some metals in soils are comparable to their contents of soils in other countries (Table 1). Main sources of metals in soils are atmospheric emissions, of short and long range of transport.

Table 1. Trace metals in various surface soils (average values, mg kg^{-1})

Metal	Locality	Light and medium light sandy soils	Medium and heavy loamy soils
Cd	World	0.40	0.52
	Europe	0.50	0.60
	Poland[a]	0.22	0.43
Cu	World	13	23
	Europe	9	24
	Poland	10	17
Ni	World	13	26
	Europe	10	28
	Poland	14	19
Pb	World	22	28
	Europe	19	33
	Poland	14	21
Zn	World	45	60
	Europe	41	46
	Poland	42	75

[a] Data for soils of Poland are for the period 1993/96, N=885.

Spatial distribution of trace metals in soils indicates a significant relation with soil textural groups. Trace metal levels are always higher in soils with higher contents of fine granulometric fraction than in sandy soils (Table 1). In some regions, however, subjected to larger loads of metals, either from atmospheric depositions or from other sources of pollution (municipal, agricultural) increased of metal contents is observed in all kinds of soils. Those regions are located mainly in south western part of the country, and also are associated with urban areas. Example of the most polluted region is Silesia District, where soils of the vicinity of the Zn-Pb smelter contain (in mg kg^{-1}) up to: Cd 143, Pb 8200, and Zn 1350. Also urban soils have elevated metal concentrations, in some sites up to: Cd 10, Zn 14000, Pb 230 (Kabata-Pendias and Pendias,1993). Those soils, however, do not account for more than about 0.3% of arable land, and only 0.1% of the soils should be excluded from the agricultural use (Terelak et al.,1997).

3. Metals in crop plants

Relatively low metal contents of soils of the most of the territory of Poland are reflected in low average concentrations in crop plants (Table 2). These data clearly indicate that metal pollution of soils and plants are only local problems, associated mainly with industrial regions.

Table 2. Trace metals in some crop plants, geometric mean (mg kg^{-1} air dried)

Plant	Cd	Zn	Pb	Cu	Ni
Cereal grain (6,500)[a]	0.06	33.3	0.23	0.44	3.74
Potato tubers[b] (5,680)	0.11	19.9	0.33	0.61	4.46
Grass (3,760)	0.12	31.6	0.89	0.84	5.51

[a] In parentheses number of samples collected in the period 1992/96 (Terelak et al.,1997).
[b] Peeled tubers.

4. Soil remediation and land use program

The assessment of soil quality for agriculture, based on metal status, has been a broad national program. Special strategies are undertaken for the reclamation of degraded soils of sensitive industrial areas. One of the most urgent problems is sewage sludge disposal, Thus current actions obtain researches on suitability of sludges for the remediation of contaminated soils (Stuczynski et al., 1998).

5. Conclusions

Trace metal levels in soils and in crop plants, not affected directly by industrial emissions and/or other loads, are lower or comparable to those found in World soils and plants. Serious impacts on local lands have been related mainly to old (and partly to contemporary) mining and smelting plants. Trace metal issues became to be a concern not only of big factories but also of small enterprises and municipal activities. These problems, however, are associated only with "hot spots", and especially in sensitive industrial areas.

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SOIL CONTAMINATION ISSUES IN THE REPUBLIC OF KOREA

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1. Introduction

The Republic of Korea, occupying the southern portion of the Korean Peninsula in East Asia, covers an area of 99000 km^2. As one of the most densely populated countries of Asia, it had a population estimated in 1995 at 45 million.

Geologically, the Korean Peninsula largely consists of Precambrian rocks such as granite and gneiss. Although granite and gneiss, which are acidic in nature, cover two-thirds of the country, Korea has a relatively wide variety of soils because of its complex topography. Results of detailed soil surveys have identified 375 soil series in Korea.

Korean soils are vulnerable to contamination due to their physico-chemical properties and topography. Among the many soil contaminants, heavy metals are particularly hazardous. Once a soil is contaminated with heavy metals, decontamination is very difficult. This paper deals mainly with soil contamination by heavy metals.

2. Background values of heavy metals in rural soils

The background values of heavy metals in Korean soils are shown in Table 1.

Table 1. Mean background values (mg kg^{-1}) of heavy metals in Korean soils.

Region	No. of samples	As	Cd	Cu	Hg	Pb	Zn
rice paddy	737	0.56	0.13	4.08	-	4.99	4.13
upland	215	0.49	0.16	3.06	0.09	4.16	-
forest	240	-	0.01	1.98	-	5.52	4.08

A large quantity of both groundwater and effluent is generally used to irrigate rice paddy soils through the growing season. Applications of effluent water rich in contaminants can lead to accumulation of both heavy metals and toxic organic substances in paddy soils. Much less irrigation water is supplied to cultivated upland soils than to rice paddy soils, but heavy metals can be accumulated in cultivated upland soils when they are used for horticulture as large amounts of fertilizers are often applied to the soils. Moreover, some heavy metals are more phytoavailable under aerated conditions compared to waterlogged reducing conditions generated in paddy soils. Heavy metals may also be accumulated in cultivated upland soils used for horticulture because there is less irrigation water and consequently less percolation and leaching losses compared to rice paddy soils. The background values of heavy metals in rice paddy soils and cultivated upland soils from the same region and the same parent material can thus be quite different.

3. Current methods of assessment used for contaminated soils

Under the Soil Preservation Act of 1995, Korean government prepared the Enforcement Decree for the Act and assesses the contamination of the soil based on it since 1996.

4. Present status of soil contamination by heavy metals

The Ministry of Environment established a soil contamination monitoring network in 1986. Based on major river systems, the network divides Korea into six environment spheres with 250 monitoring sites. Generally, heavy metal concentrations of soils from the agricultural areas were the lowest and were close to the background values. But heavy metal contents in the soils from areas around metal mining sites, smelters, and landfills for industrial wastes were generally higher than those from the agricultural areas. Zinc and Cd concentrations in rice paddy soils and brown rice around old zinc mining sites were very high compared to those in uncontaminated rice paddy soils. Rice paddy soils can also be contaminated by As from mining wastes, mining wastewater, and dusts produced in the process of mining Au, Cu and Pb. With increased air pollution by heavy metals, chances of heavy metal accumulation in soils near roadsides or in the plants from these soils are high compared to background concentrations in the forest soils. Levels of Pb contamination at the roadside are, however, expected to decrease with increasing unleaded gasoline consumption.

5. Conclusions

Establishment of standardized methods of soil analysis for soil contamination of Korea is very important. It would also be useful to establish conversion factors so that values obtained by one method can be compared with values obtained by another method. Other problems that must be resolved include determination of the number of sites necessary for a soil contamination monitoring network, and appropriate methodology. In addition, research related to soil environment management should be developed further and related to future preservation of global environments.

BRAZIL: A NATIONAL REPORT ON ENVIRONMENTAL ISSUES ON TRACE ELEMENTS

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1. Introduction

The continental Brazil has 8,547 Mm^2, allowing its 26 states to have a diverse climate, geology, and vegetation. These distinct characteristics result into several morphoclimatic and phytogeographical domains, and as a consequence of this diversity, five social-economical regions were established. Nowadays, from its 160 million population, 78% tend to be concentrated in large cities. This is the result of a transformation suffered by the economy in the last 20 years, which was changed from a primary production to an emergent industrial power status. Among the ten largest economies in the globe, 49% of this Brazilian Gross Domestic Product in 1995 was based on services, 38% on industry, and 13% on primary activities such as mining, forestry, and agriculture. Today, Brazil has a distinguished position as a primary producer and is among the world major exporters of some minerals (iron ore), ag-commodities (soybean meal, orange juice, and coffee), and manufactured products (motor vehicle parts). All these activities result in the production of different kinds of wastes which are potential sources of contaminants. In this paper, an overview about trace elements issues relating natural resources, human activities, and their environmental impacts in Brazil will be presented. Emphasis will be placed on food and water quality. An attempt to assemble a broad range of trace elements information from the most contributing sectors will be presented.

2. Sources of environmental contamination in Brazil

The major soil groups found in the Brazilian territory are oxisols, ultisols, alfisols, and inceptisols. These soils have different degrees of participation of the Brazilian regions and are distinctly subject to actual or potential sources of contamination by trace elements. Such contaminant sources could be classified in two major groups of contaminated materials: a) the purposely applied; and b) the non-purposely applied depositions. In the first group the major sources applied to both agricultural and non-agricultural sites are fertilizers, pesticides, limestone, and farm, urban, and industrial recyclable residues such as manures, municipal solid wastes, and sewage-sludge, as well as irrigation water. In the second group are sediments originated from unpaved roads, fuel emissions, mining activities and industrial plants (eg. cement and metallurgy). Many of these potential sources have been neither quantified concerning to their contribution to the soil contamination or background levels, nor even considered. Hydropower, biomass, coal, residues, and nuclear power energy production can also be important pathways for environmental contamination.

3. Nature of contaminants in agricultural soils and impacts on water and food quality

There is an increasing concern about the contribution of mineral and organic fertilizers, limestone, and pesticides used as agricultural inputs in Brazil for the soil contamination with trace elements such as Cd, Pb, Hg, and As, or even with micronutrients as Mo, B, Zn, and Cu. Few studies have detailed trace elements regulatory mechanisms of their bioavailability, and fate in Brazilian environments. Recent surveys have been conducted in more developed states such as Paraná demonstrating spacial distribution and correlation with anthropogenic activities. The soil is an important door for food chain contamination. However, because great part of the Brazilian territory is influenced by high intensity rains, contaminants are expected to be transported to aquatic environments where some trace elements could potentially become more harmful. Today, because of human pressure exerted by the growing urban concentrations, governmental policies have put great emphasis on urban residues recycling programs through agricultural systems. Besides, the private industrial sector have also seen soil recycling as an economical and environmental sound way to eliminate their residues. On the other hand, due to the low availability of some trace elements considered as micronutrients, extensive work have been conducted towards obtaining sources, minimum and toxic soil application rates for several crops in Brazil.

4. Typical concentrations in Brazilian natural and anthropogenic environments

Several geochemical surveys have been conducted in the Brazilian territory by government and private companies towards mineral prospecting. These and other author results have demonstrated that different total and extractable background levels should be expected for different geological formations, soils, and sediments. Besides, also for agricultural and forestry purposes, soil background levels have been also surveyed. However, because of the extension and diversity of the Brazilian territory, such numbers can frequently represent only regional particularities which need sub-regional representation schemes. Interestingly, some native organisms have been identified as bioaccumulators of some trace elements. On the other hand, because of concerns about water quality, some communities are conducting geochemical surveys in urban areas under the influence of disposal sites such as water bodies, landfarms, landfills, and major industrial sites such as the cases of São Paulo and Paraná States. Such studies are the result of community claims that have been attended by governmental grants. Additionally, accidental contamination cases of urban and industrial sites with trace elements are not rare.

5. Food and environmental quality assessment and remediation programs

Geostatistical as well as simpler sampling techniques have been used for soil, soil solution, and plant. Extraction and total methods recommended by different authors have been adopted. Biological indicators such as urban trees have been studied for environmental quality evaluation. Techniques such as atomic absorption spectroscopy, emission spectroscopy, inductive coupled plasma, and X-ray diffraction have been used as determination methods. At this time great emphasis has been put on trace metals „status quo" survey. Remediation strategies seems to be a consequence of this first step. In a medium run, food quality monitoring programs appear to be a necessity. Several physical, chemical and biological remediation techniques seems to have potential application, but more information is required to allow economical and effective field clean-up implementation. Considerations to support regulation purposes have been made to attend different situations.

ENVIRONMENTAL ISSUES OF TRACE ELEMENTS IN RUSSIA

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1. Introduction

According to standards set by the Russian Federation (RF), trace metals (TM) such as Cd, Hg, Pb, Se and Zn are major pollutants of the biosphere. Therefore, the scale of trace element discharge into the environment and their behavior and role in the environment have important theoretical and practical interest. This interest is heighened by the possible transborder transfer of TM from aerosols as well as by water flux. The aim of the present report is to analyse the extent and effects of environmental pollution from trace metal compounds in Russia.

2. Materials and Methods

Both published literature (Snakin, 1997) and our own data (Mocik and Pinsky, 1991) concerning the extent and effects of trace metal pollution of the environment were used for this analysis. Different environmental components: soils, surface and ground water, atmospheric aerosols, plants and animals, were analysed. The influence of trace metals on human health was also analyzed. A wide range of techniques were used for each cases (Mocik and Pinsky, 1991; Snakin, 1997).

3. Results and Discussion

The average population density of Russia is 8.63 residents/km^2. However, the population density of the European part, occupying about 25% of the total territory, is about 200 residents/km^2. In the rest of the RF, this index varies from 1 to 10 residents/km^2. Most of the industry and agriculture in Russia is confind to the more densely populated regions.

The soils and natural waters in the RF reveals little or no wide-range TM contamination from industrial sources. Nevertheless, there is regional pollution near large industrial centers, cities and natural geochemical sources. The territories closely adjacent to the sources are subject to the highest contamination. Thus, the content of Zn, Pb and Cu in soils not far from the Mid-Ural copper smelting plant reach 80, 500 and 2859 mg/kg, respectively. Depending on the direction from the plant, the concentrations of these TM decrease to background levels at distances of 11-20 km. These levels are still 1.5 to 2 times higher than the average values for analogous soils in the European region of the RF and are characteristic of the natural geochemical conditions of the Mid-Ural region.

The concentration of TM in agricultural and forest soils does not exceed the values for the maximal permissible concentrations (MPC) set by. The only exception is in agricultural soils on which high rates of sewage sludges have been used as fertilizer. The soil concentration of trace metals in most cities of the RF is higher than in agricultural or forest soils but still fall within the range for MPC. However, in some industrial centers, in particular, Rostov-on-Don, Mednogorsk, Svirsk and others, concentrations can exceed the MPC by as much as 10-fold.

The level of contamination of soils, surface waters and atmosphere from trace metals from sources within the RF has steadily declined in recent years for two major reasons: reduced industrial production since 1991 and the improvement in waste water treatment. The variation in the metal's

concentration can be demonstrated by measures of the Pb concentration in the largest rivers of the RF: the Don - 1.5 to 6.5 mg/L, the Volga - 1 to 11 mg/L, the Ob - 2-18 mg/L, and the Enisei - 2 to 19 mg/L. The levels are all significantly below the values of MPC adopted in Russia for drinking water. One exception is the Amur River, which shows a Pb concentration range 1.5 to 3 times higher than the MPC. As expected, the TM concentrations in ground and surface water near mines of polymetallic ores may exceed the MPC by 10-fold. Furthermore, the concentrations of suspended TM may exceed soluble ones by 70-fold (Pryde, 1995).
The quantities of Pb discharge into the atmosphere in the different region of the RF are:

Region	% of total	Region	% of total
Yekaterinburg Region	69.0	Cheliabinnsk Region	2.5
Primorsky krai	10.0	North Osetiya Republic	2.2
Krasnoyarsky krai	4.7	All others	9.0
Moscow and Moscow region	3.2		

This is of great significance when transregional and transborder transfer of TM are considered. For Europian part of Russia the balance of Pb in 1991 at transborder transfer was estimated as +3260 tons. Most part of TM enters from the countries of Western and Eastern Europe, China and Kazakhstan.

4. Conclusions

The analysis of the levels of environmental pollution in the territory of Russia shows that there is no widespread contamination by trace metals. At the same time, local levels of trace metals in soils and natural waters indicates pollution does occur near large cities, industrial centers and natural geochemical anomalies. An analysis of the balance in the transborder transfer of trace metals into the RF demonstrates that the greatest amount enters from the countries of Western and Eastern Europe, China and Kazakhstan.

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ARSENIC IN GROUNDWATER AND SUFFERINGS OF PEOPLE IN EIGHT DISTRICTS OF WEST BENGAL, INDIA

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1. Introduction

There are approximately 20 countries where groundwater arsenic contamination episodes in the world are known but the world's four biggest calamities and worst sufferings of the people have been in Asia. In order of magnitude these are Bangladesh, West Bengal-India, Inner Mongolia, Xia-Xiang Province-P.R. China and Taiwan. In West Bengal-India eight districts - South 24-Parganas, North 24-Parganas, Nadia, Murshidabad, Malda, Hooghly and Howrah are arsenic affected. In these eight districts arsenic has been found in groundwater above maximum permissible limit (0.05 mg l^{-1}) recommended by WHO. A summary of the present arsenic status, as revealed by our survey during the last 10 years is presented in Table-1. Although 38 million people are living in 38,000 sq. km arsenic affected area, this does not mean that all are drinking arsenic contaminated water and will suffer from arsenic toxicity but no doubt they are at risk. We have reported the arsenic problem in West Bengal and Bangladesh in our earlier publication (Mandal et. al. 1998).

2. Materials and Methods

48,000 water samples were analysed from hand tubewells. About 10,000 each of urine, hair and nail samples, a few hundreds of skin-scale, breast milk and blood sample were analysed from affected area. Flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS) was used for analysis of most of the water, urine and digested biological samples.

3. Results and Discussion

So far from 8 affected districts and 920 villages of West Bengal we have collected about 48,000 water samples and measured for total dissolved arsenic. We have also measured few thousand sample for As(III) and As(V) and the average ratio of As(III) : As(V) is 1:1. About 40% of the water samples contain arsenic above 0.05 mg l^{-1}, the WHO maximum permissible limit. The highest concentration of arsenic so far recorded is 3.7 mg l^{-1}. The average concentration of arsenic in drinking water is 0.2 mg l^{-1}.

Table-2 shows and the arsenic metabolites in urine from arsenic affected areas. The results show urinary arsenic above normal level to 73% population in arsenic affected villages. Table-2 also shows elevated level of arsenic in hair and nail measured for a few thousand population. There is no recommended value of arsenic in skin scale. Higher skin scale arsenic indicates obvious body burden of arsenic. In our field study we have observed that in affected villages many people are drinking contaminated water but have no arsenical skin lesions, although their hair, nail arsenic contain high arsenic. Thus these people may be sub-clinically affected. People living in arsenic affected area can not avoid contamination as they are living in arsenic environment.

A good percentage of people have arsenic in biological samples above normal level although their tubewells are safe to drink. So far in our preliminary survey in 8 districts we had examined at random 28173 people and 4113 (14.6%) people have been found with arsenical skin lesions. People suffering from malnutrition are mostly affected.

4. Conclusions

From the study of arsenic groundwater contamination and sufferings of people it appears the overall situation is serious. More we are surveying more and more arsenic affected area and patients are coming to limelight.

What we need at this moment to combat the situation is an alternative source of water other than underground water. West Bengal has 2000 mm of average rainfall, about 4000 km^2 of wetland and vast river basins flooded almost every year. We should conserve these water resources. A proper watershed management is the need of the day. Due to our negligence most of these water bodies go dry or grow water-hyacinth. If we can use these water bodies not only villagers will get arsenic free water but using pisciculture, duckery, growing vegetables at the bank of these water bodies the economic condition of villagers will be much better. This arsenic calamity of West Bengal, which is one of the biggest in the world, should not be neglected any more. The world should also learn a lesson from this arsenic problem of West Bengal that any country where water extraction from underground goes similarly unchecked could be leaving themselves open to a similar calamity.

Table-1 Physical parameters of eight arsenic affected districts of West Bengal-India

Total area of West Bengal in sq. km	Total population of West Bengal in million	Total area of arsenic affected 8 districts of West Bengal in sq. km	Total population of arsenic affected 8 districts of West Bengal in million	Total area of 63 arsenic affected blocks of West Bengal in sq. km	Total population of 63 arsenic affected blocks of West Bengal in million	Total no. of arsenic affected villages/ wards	Population drinking arsenic contaminated water above 0.05 mg l^{-1} in million	Population showing arsenic related skin manifestation
89000	68	38000	38	10500	12	911	4.5 (approx.)	300,000 (approx.)

Table-2 Parametric presentation of arsenic in urine, hair, nail, skin-scale, blood and breast-milk samples from arsenic affected areas

Parameters	Arsenic affected victims with skin-lesions				People drinking arsenic contaminated water without skin-lesions			People drinking safe water with respect to arsenic			Blood ($\mu g\ l^{-1}$)	Breast-milk@ ($\mu g\ l^{-1}$)
	Urine* ($\mu g\ l^{-1}$)	Hair** ($mg\ kg^{-1}$)	Nail*** ($mg\ kg^{-1}$)	Skin-scale# ($mg\ kg^{-1}$)	Urine* ($\mu g\ l^{-1}$)	Hair** ($mg\ kg^{-1}$)	Nail*** ($mg\ kg^{-1}$)	Urine* ($\mu g\ l^{-1}$)	Hair** ($mg\ kg^{-1}$)	Nail*** ($mg\ kg^{-1}$)		
Sample No.	812	806	802	165	6346	4715	4786	2137	1543	1793	104	250
Mean	462	1.48	4.56	6.82	286	1.33	2.78	86.59	0.94	1.60	30	17.87
Maximum	3893	20.34	44.89	15.51	3947	6.39	21.36	149.99	1.99	2.94	145	49
Minimum	55	0.96	0.88	1.28	10	0.18	0.38	17.10	0.21	0.40	5.30	2
Median	293	1.32	3.87	4.46	184	1.16	2.31	93.75	0.83	1.46	21	15.5
Standard Deviation	579	2.20	3.98	4.75	342	0.79	2.0	39.3	0.42	0.61	26.85	12.1
% of samples having arsenic above normal/toxic level	89%	68%	79%		81%	46%	62%	48%	26%	34%		

* Normal excretion level of arsenic in urine range from 5-40 $\mu g\ day^{-1}$ (1.5 l)

** Normal amount of arsenic in hair is about 0.08-0.25 $mg\ kg^{-1}$ with 10 $mg\ kg^{-1}$ being the indication of toxicity

*** Normal arsenic content in nail is 0.43-1.08 $mg\ kg^{-1}$

\# There is no available data of normal level of arsenic for skin-scale in literature.

@ Out of 250 breast-milk in 40 samples arsenic has been detected.

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FLUOROSIS IN INDIA: STATE OF ART REPORT

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1. Introduction

Fluorosis, a crippling disease is known to occur in India due to ingestion of Fluoride. Unlike the Western World where there is deliberate addition of Fluoride, in India drinking water is naturally contaminated with Fluoride. Fluoride arises from the geological crust of India. An estimated 62 million people are adversely affected due to consuming Fluoride contaminated water of which 6 million are children below the age of 14 years. There are 22 other nations around the Globe where Fluorosis is a Public Health problem. The objective of this report is to (1) reveal the type of networking required to combat the problem of Fluorosis (2) specify the technologies adopted in India for removal of Fluoride from drinking water (3) specify the importance of base-line health and water quality surveys (4) categorize the water quality test data (5) highlight the interventions practiced to prevent the disease (6) reveal the clinical modules and diagnostic tests required to be carried out for early detection of Fluorosis (7) comment on the out-dated, unscientific concept of use of Fluoride for prevention of Caries, promoted in the developed world.

Networking to combat Fluorosis

Fluorosis control is not the domain of a single agency / Ministry in the Government. As the control programme of Fluorosis, require expertise from diverse disciplines it is necessary to identify those who have the skills required for programme implementation at the planning stage of the programme itself (Susheela and Ghosh, 1998).

Technologies adopted for removal of Fluoride from drinking water

In India, in rural areas, drinking water is collected by the community from wherever water is available. Surface water sources were used until mid 1970s, when epidemics of Cholera, Typhoid, Jaundice and other water-borne diseases, were too frequent. In order to control such epidemics, deep tube wells were dug considering that water from deeper aquifers are safe. The tube well water culture has been since developed in India. There are 1,16,500 mini-pipe water supply schemes of which 50% of them are based on tube wells. Besides there are 30 lakh hand pumps used for drifting underground water sources. As tube well waters are contaminated with Fluoride, it became mandatory to remove the Fluoride to make it potable. The Nalgonda technology using alum and lime to flocculate Fluoride is recommended. Besides, activated alumina is used as an adsorbent for Fluoride and safe water is obtained. Provisions for obtaining water through community installations, hand pump attachment and domestic filters are available (Balusu, 1993, Venkobacher & Leela Iyanger 1996).

Importance of base line survey on Health & Water quality

It is important that the programme implementation should commence with a base-line survey for Health and Water quality. Both the surveys should be conducted by personnel who have been up-dated on recent developments in the field. Water quality testing for Fluoride should be carried out by modern methods, using Ion selective electrode technology. These precautions need to be taken, because Fluoride as low as possible in drinking water is preferred; and not to exceed 1 mg/l in tropical countries (Susheela, 1993).

Categorization of the survey data

It is extremely important to categorize the survey data, for programme implementation and for provision of safe drinking water on a sustainable basis. Safe water sources, if any, should be used as the best approach. If there are no safe sources of water, the possibility of bringing water from the neighborhood should be explored, if the proposal is economically viable. The third alternative is to consider rainwater harvesting, if the rainfall is adequate. The last alternative is chemical treatment using Activated alumina as an adsorbent or by using Nalgonda technology using lime and alum for flocculating Fluoride. Different modes for delivery of water, i.e. domestic filter, hand pump attachment and community installations are possible and the option / choice should be left to the community depending upon their ability to contribute towards the cost and maintenance.

Interventions practiced for control of Fluorosis

The two major interventions practiced in India for Fluorosis Control are (1) Provision of safe water on a sustainable basis and (2) Nutritional intervention with emphasis on adequate in take of calcium, vitamin C and other anti-oxidants. The interventions yield beneficial results if the disease is detected early and interventions commence soon after detection. There is a need to monitor those at frequent intervals to achieve the best results.

Clinical modules for Medical Professionals

As several health problems arise due to Fluoride ingestion, which has overlapping manifestations with other diseases, modules have been developed to alert the General Medical Practitioners in their clinical practice. If the health complaints suggest the possibility of overlapping manifestations, testing of Fluoride is to be carried out in blood, urine and drinking water to confirm whether the disease is Fluorosis or not. This approach is recommended for early detection of Fluorosis.

The approach for preventing Dental Caries

The developing world should resist from promoting Fluoride for prevention of Dental Caries. Scientific evidences suggest that Fluoride aggravate cavity formation, as calcium is lost and de-mineralizes the teeth. The practice of promoting oral health and hygiene with adequate calcium and vitamin C in the diet prevents Caries. Use of Fluoride for inhibiting bacterial enzymes to prevent acid production, though valid is contributing to more serious health problems due to the same Fluoride inhibiting tissue enzymes, deranging metabolism leading to a variety of health problems (Susheela, 1998).

Conclusion

An integrated approach involving Public Health Engineering and Health Sector Personnel working in a synchronized manner is recommended to bring out the best results to combat Fluorosis.

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HEAVY METAL POLLUTION OF SOILS AND WATER AND THEIR TRANSFER INTO PLANTS IN BANGLADESH

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1. Introduction

There are about 30000 industrial units particularly in and around the cities of Bangladesh. Their wastes effluents contain organic toxins and heavy metals especially Cr, Zn, Pb, Cd, Hg, Ni, As and Mn, which pollute water system and agricultural lands. These metals and toxic substances are taken up by plants and thereby enter into food cycles causing health hazards (DOE, 1992).
A research program financed by the Austrian Academy of Science has been undertaken to investigate the extent of pollution of soils and water particularly with heavy metals discharged from the factories and their transfer coefficients into agricultural crops. Six industrial sites were selected for investigation. They were Hazaribagh Tannery, Tongi battery and pharmaceutical, Tejgaon industrial, Narayanganj textile and dyeing and Pagla sewage treatment areas. Hazaribagh, is heavily polluted with hazardous wastes and effluents of 145 tannery factories discharging 40t solid wastes and using 100 kg Cr/ day. Tongi industrial area is about 18 km north of Dhaka City and is largest industrial complex in Bangladesh with an area of 16 km^2. The battery factory is in operation since 1962 and pharmaceutical since 1982 in this area. Tejgaon is the largest industrial zone within Dhaka City influenced by sanitary, battery, soap, printing and dyeing, casting, electroplating, rubber and plastic, utensil, biscuits and some metal work industries. Dholai Khal is situated in the old city. This area is contaminated with heavy metals from metal works, few industries and city waste. Pagla is to in the southeastern region of Dhaka City, where the sewage of the city dwellers is treated and the effluents are discharged into the river Buriganges after treatments. Narayanganj is situated to the southeast of Dhaka City and is influenced by jute and textile industries. This area is heavily polluted with organic toxins and metals used in dyes. Both Pagla and Narayanganj are within Dhaka-Narayangonj-Demra irrigation project area and are never flooded.

2. Research Program of Heavy Metal Pollution

Within this research program, soil, water, effluents, agricultural crops and aquatic plants were investigated for their quality. Soils were analyzed for their physical and chemical properties and metal contents. Plant samples were investigated to assess the transfer coefficient of heavy metals into plants in order to assess the extent of metal pollution in the wastes influenced areas.

3. Results and Discussion

The soils of Hazaribagh, Dolai Khal, Tongi, Tejgaon, Pagla and Narayanganj were investigated. The soils of these sites differed in their physical and chemical properties. High pH values were obtained at Hazaribag up to 8.1. At Tejgaon and Tongi the pH was around neutrality. Very low pH was obtained at Pagla and Narayanganj (4.5 and 4.8 respectively). Although very high pH values were obtained in contaminated water and effluent in Hazaribagh and Tejgaon, soil pH was not much influenced probably due to higher buffering capacity resulting from high content of organic matter or neutralization of the systems by the alternate release of acids and alkalis from the factories. Electrical conductivity, CEC, OM, N and chloride contents were also influenced by the wastes and effluents of the factories.
Hazaribagh tannery area is characterized by very high contents of Cr, Zn and Cu, while Tongi battery factory site has very high levels of Zn and in few cases Hg. Tejgaon industrial area is excessively polluted with Cd (163 mg/kg) and Ni (194 mg/kg). Narayanganj soil is heavily contaminated with Ni (146mg/kg), Pb (up to 185mg/kg) and sometimes with Cr. Pagla soils were contaminated with Ni (201mg/kg) and Pb (125 mg/kg) (Table-1). The heavy metals were high in grasses, waterhyacinth and rice plants adjacent to industries. Grass

accumulated more and tolerated higher amounts of heavy metals than those of waterhyacinth and rice. Chromium concentration was low in grains and straw compared to those of soils. Rice accumulated more metals in roots than in straws. Experiments with rice, wheat and lettuce grown on contaminated soils of Tejgaon showed that rice, although grow luxuriantly, gave significantly lower grain yields. Lettuce growth was, on the other hand, severely affected by the toxicity of heavy metals present in soils, which in some cases caused the death. Cadmium and nickel were found in toxic concentration in all three crops. Lettuce absorbed and accumulated the highest amount of heavy metals in roots and shoots followed by wheat. The uptakes of metals were lowest in rice plants.

The transfer coefficients of rice, wheat and some vegetables were investigated for Cr, Zn, Cu, Hg, Mn, Cd, Pb, and Ni. In general vegetables particularly lettuce have the highest transfer coefficient. The transfer coefficient of Zn, Cu and Mn to rice plants were higher than waterhyacinth in Hazaribagh tannery area. Chromium was high in grass but low in rice and waterhyacinth. In all cases, transfer of heavy metals into waterhyacinth was the lowest of the plants studied. The metal transfer into rice plants followed the trend: Cd>Mn>Zn>Cu>Hg. Mercury and copper transfer into rice plants exceeded the literature values (Schreimelechner *et al.* 1991). Metal contaminations of soils, sediments water bodies and plants have also been reported earlier (Bharma and Costa, 1992; Ullah *et al.,* 1995; Venkatesh and Abhimannyu, 1998).

4. Conclusions

The investigated sites were heavily polluted with metals discharged from the industries and their surroundings. The Hazaribagh tannery area was highly contaminated with Cr, Zn, and Cu, while Tongi battery factory site particularly with Zn, Tejgaon industrial area with Ni and Cd and Narayanganj with Pb and Ni. Heavy metal contents were very high in grasses, waterhyacinth, rice and vegetables close to the factories. The transfer coefficients were the highest in lettuce followed by rice in most cases. Such entry of metals into food chain through plant uptake might cause health hazards and also environmental problems.

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Table - 1 Metal concentrations (mg/kg) in soils of different industrial sites within and around Dhaka City.

LOCATION	Cu	Mn	Zn	As	Ni	Cr	Hg	Pb	Cd
Hazaribagh	115	263	330	2.13	25	25014	0.17	44.2	Tr
Tongi (Batteryfact)	27.3	476	2026	2.60	37	48.3	3.41	19.6	0.32
Tejgaon	47	119	200	2.81	194	54.1	0.63	70.0	163
Shawan (N. ganj)	60	371	109	3.15	85	75	1081	174	0.70
Kanak (N. ganj)	50	362	168	3.68	94	109	2.15	171	0.50
Enayetnagar (N. ganj)	47	392	142	5021	146	110	1.63	185	0.70
Bholail (N. ganj)	53	534	116	4.27	84	92	1.49	183	0.60
Pagla :	25	550	525	3.35	201	Tr	Tr	725	Tr

Trace Elements in Biosolids and other Wastes

(Special Symposium 8)

SOURCES OF HEAVY METALS TO AGRICULTURAL SOILS IN ENGLAND AND WALES

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1. Introduction

Heavy metal additions to agricultural soils can occur through applications of organic manures, fertilisers and lime, industrial 'wastes', agrochemicals, irrigation water and via atmospheric deposition. In view of concerns about heavy metal impacts on soil fertility and the potential transfer of certain metals (in particular Cd and Pb) to the human foodchain, it is important to quantify these inputs and to assess which soils are most vulnerable to further pollution.

2. Methodology

An inventory of metal inputs to agricultural soils in England and Wales was constructed for Zn, Cu, Ni, Pb, Cd and Cr from the following major sources :

- livestock manures
- sewage sludge
- industrial 'wastes'
- atmospheric deposition
- fertilisers and lime
- agrochemicals
- irrigation water

The methodology used to calculate the total heavy metal inputs and typical loading rates from each source was described by Nicholson *et al.*, 1998. These were used to estimate the time it would take to increase topsoil concentrations from background (McGrath & Loveland, 1992) to the limit values where sewage sludge is applied to agricultural land (DoE, 1996). Following this, farm census data on animal numbers, estimates of manure production and manure metal concentrations (Nicholson *et al.*, in press) were combined in a GIS mapping system, to create maps of heavy metal loadings from farm manures, to identify areas of England and Wales where elevated loadings were occurring.

3. Results and Discussion

For Zn, Cu and Ni, 25-39% of total annual inputs to agricultural land were from animal manures, 32-45% from atmospheric deposition and 7-15% from sewage sludge. By contrast, 72% of Pb was from atmospheric deposition and only 8% from animal manures. For Cd, 51% of inputs were from atmospheric deposition and 34% from fertilisers (mainly phosphates) and lime, with 11% from animal manures. The major source of Cr to agricultural land was industrial 'wastes' (38%), with sewage sludge, fertilisers and lime, and atmospheric deposition all of similar importance (c. 20%). Although atmospheric deposition and animal manure applications were generally the major sources of heavy metal inputs to agricultural land in terms of total quantities, loading rates to individual fields were relatively low. By contrast, metal loading rates for fields receiving sewage sludge or industrial wastes were usually higher than from livestock manures.

Estimates suggested that it would take c.150 years to raise topsoils from background to Zn limit concentrations if de-inked paper crumble was applied annually at typical application rates, and 150 years for sewage sludge and 200-300 years for pig or laying hen manures applied annually at rates of 250 kg/ha total N (Figure 1). These times would be decreased if soil Zn concentrations were already above background levels, if more than one material was applied to a field or if application rates were higher. However, it would take >3000 years for atmospheric deposition alone to raise topsoils Zn to limit concentrations. Similar estimates have also been produced for the other metals.

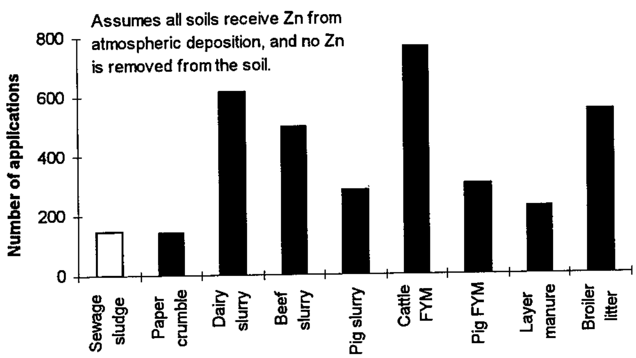

Figure 1 : Number of applications required to raise soil Zn concentrations from background (80 mg/kg) to limit (300 mg/kg) levels.

GIS maps of *partial* metal fluxes for rural agricultural soils were produced including data on inputs with atmospheric deposition, fertilisers and livestock manures, and offtakes in harvested crops. These indicated that the highest net inputs of Zn and Cu were associated principally with the main pig and poultry farming areas of England and Wales.

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RELATIONSHIP BETWEEN SOIL COPPER ADSORPTION AND DOM DERIVED FROM SEWEAGE SLUDGE AND COMPOST

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1.Introduction

Interaction of Cu with dissolved organic matter (DOM) could affect greatly the metal transport and mobility in soil leading to potential environmental problems (Li and Shuman, 1996). The incorporation of sludge and compost to soils would increase the amount of DOM due to the enriched organic matter in these waste materials (Baham and Sposito, 1983). It is the objective of the present study to investigate the effect of sludge and compost DOM on Cu adsorption and to elucidate major factors such as DOM concentration, chemical fractions, and pH affecting their interaction.

2.Materials and Methods

Dissolved organic matter was obtained by extracting dewatered anaerobically digested sludge and sludge compost with deionized water at 20±1°C for 16hr. The DOM was fractionated into hydrophilic and hydrophobic acid, neutral, and base (Leenheer, 1981). Copper adsorption isotherms for the two soils were performed with or without the addition of 400 (0 to 400?)mg C L^{-1} of sludge or compost DOM at 20±1°C. The effect of DOM concentration (0 to 800 mg L^{-1}) and pH (pH 2 to 10) on Cu adsorption were also studied using an initial Cu concentration of 40 mg L^{-1}.

3.Results and Discussion

The acid fractions (hydrophilic and hydrophobic) are the dominant fraction accounting for 78% and 73% of total DOM of sludge and compost, respectively (Table 1). Compost DOM had a higher hydrophobic fraction than that of sludge DOM. There was an increase in hydrophobic neutral and hydrophobic acid but a decrease in hydrophilic acid after composting. This implies that the DOM in sludge would be more chemically active than that of sludge compost.

Copper adsorption on the two soils were significantly reduced at a DOM concentration of 50 mg CL^{-1} derived from both sludge and compost, with a higher reduction for DOM from sewage sludge (32%) than that from sludge compost (20%) (Figure 1). From the Freundlich adsorption model, the equilibrium partition coefficient (k) was smaller, while the binding intensity parameter (1/n) was larger in the presence of sludge DOM than that of compost DOM for both soils. This indicated that the soil binding capacity of both soils for Cu decreased to a greater extent for sludge DOM than that of compost DOM (Table 2). An increase in DOM concentration significantly reduced the adsorption of Cu by both soils, and the reduction was higher for sludge DOM than that of compost DOM. This can be attributed to the higher hydrophobic fraction in compost DOM. In addition, DOM caused a relatively stronger reduction in Cu adsorption capacity for both soils with an increase in soil pH (Figure 2). This is due to the stronger bonding strength between Cu and DOM at high pH than that in low pH condition.

Table 1. Distribution of hydrophobic and hydrophilic fraction of DOM in sludge and compost.

Components	Sludge	Compost
Total DOM	100	100
Hydrophobic acid	38.5a	52.0a
Hydrophobic base	0.81d	0.43d
Hydrophobic neutral	0.85d	22.0b
Hydrophilic acid	39.4a	21.2b
Hydrophilic base	16.2b	2.57c
Hydrophilic neutral	4.18c	1.86cd

Table 2. Freundlich equilibrium constants for Cu adsorption by the soils with or without the addition of DOM.

	Acidic sandy loam			Calcareous loam		
	No DOM	Sludge DOM	Compost DOM	No DOM	Sludge DOM	Compost DOM
K	52.1	10.7	32.1	2776	47.9	379
1/n	0.79	1.00	0.85	0.30	0.87	0.55
$R^{2\xi}$	0.98	1.00	0.99	0.94	0.93	0.96

$^{\xi}$Significant at p<0.01.

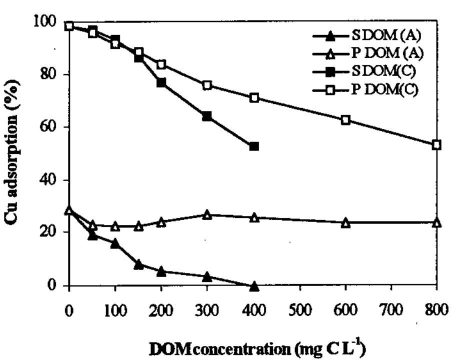

Figure 1. Effect of various concentrations of sludge (S) and compost (P) DOM on Cu adsorption on acidic (A) and calcareous (C) soils.

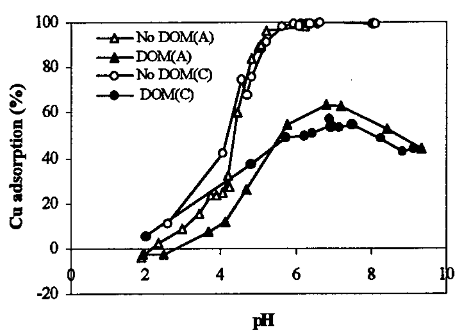

Figure 2. Effect of pH on Cu adsorption onto acidic soil (A) and calcareous soil (C) with and without the addition of 300 mg C/L of sludge DOM.

4.Conclusions

The present study showed that addition of DOM derived from both sludge and sludge compost significantly reduced the soil adsorption capacity for Cu especially for raw sludge. Therefore, heavy application of sewage sludge may lead to the downward movement of Cu from topsoil.

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CHARACTERIZATION OF TRANSITION METAL SPECIES IN THE SOLUTION OF SANDY SOIL AMENDED WITH SEWAGES SLUDGE

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1. Introduction

Application of sewage sludge to improve physicochemical properties of arid-zone soils may result in the release to the environment of heavy metals. The soluble fraction accounts usually for only 0.5-7% of the total amount of sludge borne heavy metals (Lake et al. 1984, Liang et al. 1991), but it is a major contribution to the total metals transported through the soil or taken up by plants following sludge application.

Transition metals in the aqueous phase of soil or bio-solids, can apear as free ions or as complexes with organic or inorganic ligands. The metal type and environmental conditions such as pH, ionic strength and potential ligands concentration determine the form of the metal species. Stability constants of transition metal complexes were determined in natural and artificial solutions in the past (Stevenson and Fitch 1986, Mantoura et al. 1978, Stevenson and Chen 1991). Copper, iron, nickel and lead were found to have a strong tendency to form complexes whereas cadmium was a week complexant. Zinc, cobalt and manganese displayed a moderate tendency to form complexes, and their stability constant values were very much affected by the environmental conditions.

The objectives of the study were to characterize heavy metals species in the soluble fraction of sewage sludge according to charge, species molecular weight and the complex structure, to study the changes in metal speciation with time after sludge application to soil and to define the complexation capacity of the water extracts of sludge or soil-sludge mixtures.

2. Materials and Methods

Distribution of the species of the trace elements in the aqueous extract of sewage sludge according to their charge was estimated by using a combination of cationic and anionic ion exchangers (Dowex-50W acidic cation exchanger, Dowex macroporus resin-basic anionic exchanger). The molecular weight distribution was determined by gel chromatography (Sephadex G-25-80). Complexation Capacity (CC - the maximum load of transition metals that potential ligands in the aqueous phase can form complexes with) of the sludge extract was also determined by gel chromatography using diluted sludge extracts (sludge:water ratio - based on weight - 2:25, 1:50 and 1:100). CC was estimated from the total Cu content of the leachates of the gel chromatography columns, the eluents of which were fortifed with excess Cu, less the content of the free Cu as deduced from the area of the free Cu peak in the chromatograms. Changes in speciation with time of the water extractable fraction of the metals was studied in mixtures of 1%-20% sludge with soil, incubated for a period of 100 days. Pure sludge and soil samples were also studied. The properties of the water extracts of the incubated samples were determined periodically. Characterization of the molecular weight and charge of the transition metals in the extract was carried out during the incubation.

3. Results and Discussion

Sludge addition to soil increased the concentration of soluble metal species and Soluble Organic Carbon (SOC) dramatically. Yet, these concentrations decreased by half or more

during the 100 days incubation of the soil-sludge mixture. The soluble organic carbon is characterized by two dominant molecular weight fractions of 300-1000 and 1500-4000 Dalton. The concentration of the high molecular weight fraction of SOC decreased dramatically during the first month of incubation while the low molecular weight fraction decreased to a considerably lower extent. Most of the soluble Cu species had a molecular weight in the range of 300-1000. As the incubation proceeded, the capacity of the water extract of the soil-sludge mixtures to complex Cu did not change significantly. Also, there was no significant change in the distribution of the Cu species according to charge during the incubation. A moderate decrease in the fraction of soluble Zn found in zwitter ion form or in species possessing easily reversed charge and an increase in the fraction of positively charged Zn species were observed during incubation.The complexation capacity of the aqueous extract of sewage sludge for Cu was found to be between 6.5-18 mM/g SOC. The complexation capacity values in the relevant literature range between 0.01-8.4 mM/g SOC for natural water, effluent or sludge. However, it is difficult to find in those references significant correlation between the solution origin and the complexation capacity. The average number of ligands attached to a metal ion was estimated based on the complexation capacities and the molecular weights of the complex and the ligands. The Cu to attached ligands ratio (Cu:ligand) was found to be 4:1 in the diluted sludge extract and 3:5 in the concentrated extract. Since all the extracts were fortified with a fixed concentration of free Cu, not enough free Cu was available to saturate all the complexation capacity in the concentrated extract and complexes were formed mostly with high binding affinity ligands. In the diluted extracts, weaker ligands were also bound to Cu. The average number of carbons in the ligand chains attached to Cu was 12-15 and 6-7 in the diluted and concentrated extracts, respectively.

4. Conclusions

The addition of sludge to soils may result in the release into solution of both organic and inorganic anionic species, which may serve as potential ligands. This release may continue over an extended period. Such ligands are capable of rendering considerable quantities of Cu and most likely other metals, soluble and mobile. The availability of sludge borne metals, both to plants and to downward transport, is considerably higher than that of metal ions added to soils as mineral salts. The higher the tendency of the metal ion to form soluble complexes, the higher is the mobility of the sludge born metals. The soluble Cu in the water extract of the sewage sludge-dune sand mixture was dominated by low molecular weight complexes (MW below 1000). Higher molecular weight SOC which displayed a concentration peak around MW=2000, did not form complexes with Cu to a significant extent. Even when soluble organic carbon concentration declined by more then 50% (after incubation for 100 days of all sludge - soil mixtures), the complexation capacity of the water extract of the mixtures for Cu remained high, supporting the assumption that ligands come predominantly from a specific (the more stable, low MW) fraction of the SOC.

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FACTORS AFFECTING THE SOLUBILITY OF ZINC, CADMIUM, COPPER AND NICKEL IN SEWAGE SLUDGE AMENDED SOILS

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1. Introduction

Although current regulations on heavy metal contamination are largely based on the total metal concentrations, it is known that the soil solution concentrations of heavy metals provide a far better indication of bioavailability than the total concentrations of metals in soils (Peijnenburg et al 1997; McGrath et al 1998). The relationship between soluble and total metal concentrations in soils is governed by factors which control adsorption or precipitation of metals, such as pH, contents of organic matter and oxides (Jopony and Young 1994; McBride et al 1997; Lee et al 1996; Janssen et al 1997). This study aims to derive empirical relationships which can be used to predict concentrations of Zn, Cd, Cu and Ni in soil pore water from other soil properties such as total metal concentrations, soil organic C content, pH and dissolved organic C concentration in sewage sludge amended soils.

2. Materials and Methods

Seventy-two soils were collected from three field experiments using different types and rates of sewage sludge. The Gleadthorpe experiment was established in 1968 and the two Braunschweig experiments on arable land and ex-woodland in 1980. Fresh moist soils were sieved to <3 mm, and placed in plastic pots each containing equivalent of 1 kg dry weight. Soil moisture contents were raised to 75% of water holding capacity and left to equilibrate for two weeks. Soil solutions were extracted using Rhizon soil-moisture samplers which were made of porous plastic capped with nylon (Knight et al 1998). Metal concentrations in solutions were determined using ICP-AES or GF-AAS. Total metal concentrations were determined after aqua regia digestion, and EDTA-extractable metals following extractions with 0.1 M EDTA in a 1:5 soil to solution ratio. Other soil properties determined included soil organic C (SOC), pH (in a slurry of 1:2.5 soil to water) and dissolved organic C (DOC) in soil solution. Best-fit equations which described the relationships between soluble metal concentrations and other soil properties were obtained using step-wise regression.

3. Results and Discussion

The soils used varied considerably in pH (5.2-7.2), SOC (0.8-2.4%), DOC (13-63 mg L^{-1}) and total metal concentrations (Zn 38-444, Cd 0.3-3.3, Cu 6.6-365 and Ni 5.9-35 mg kg^{-1}). Best-fit regression equations were obtained for the prediction of soluble metal concentrations in the soil pore water using either total metal or EDTA-extractable metal concentrations, in association with pH, SOC and DOC (Tables 1 and 2). These equations accounted for a high degree of variation in the soluble metal concentrations. Use of EDTA-extractable metals instead of total metals improved the predictions for Cd and Ni. Total or EDTA-extractable metals were a significant factor controlling the concentrations of the four metals studied in the soil solutions, being particularly prominent for Cu and Zn. Soil pH was highly significant in the regression for Zn and Ni, but not for Cd and Cu when total metals were used. It became a significant factor for all metals when EDTA-extractable metals were used, and its influence followed the order of

Zn>Ni>Cd>Cu. SOC was highly significant for Cd, as well as Ni when total metals were used, and to a lesser extent for Zn, but not for Cu. In contrast, DOC played a significant role for Cu.

Table 1. Regression equations for predicting concentrations (µg L^{-1}) of soluble metals in soil pore water using total metal concentrations (mg kg^{-1}) and other soil properties. All data except pH were logarithmically transformed.

Metal	Regression equation obtained	$R^2_{adjusted}$	Variance ratio			
			Total metal	Soil pH	SOC	DOC
Zn	Y = 4.34 + 1.77 TOTAL – 0.87 pH + 1.11 SOC	0.954	1032.4***	418.6***	14.7***	NS
Cd	Y = -0.27 + 0.86 TOTAL + 4.95 SOC	0.813	25.9***	NS	267.6***	NS
Cu	Y = -1.30 + 0.98 TOTAL + 1.13 DOC	0.976	2702.3***	NS	NS	145.6***
Ni	Y = 3.54 + 2.02 TOTAL – 0.72 pH + 2.14 SOC	0.874	44.0***	59.8***	356***	NS

Table 2. Regression equations for predicting concentrations (µg L^{-1}) of soluble metals in soil pore water using EDTA extractable metal concentrations (mg kg^{-1}) and other soil properties. All data except pH were logarithmically transformed.

Metal	Regression equation obtained	$R^2_{adjusted}$	Variance ratio			
			EDTA-extractable metal	Soil pH	SOC	DOC
Zn	Y = 5.95 + 0.98 EDTA – 0.79 pH + 1.86 SOC	0.953	966.1***	418.7***	44.2***	NS
Cd	Y = 3.88 + 0.82 EDTA – 0.54 pH + 2.31 SOC	0.937	122.8***	86.5***	807.2***	NS
Cu	Y = -0.32 + 0.78 EDTA – 0.06 pH + 1.05 DOC	0.976	2711.4***	6.1*	NS	149.4***
Ni	Y = 4.59 + 1.26 EDTA – 0.71 pH + 0.73 DOC	0.940	781.5***	268.4***	NS	5.8*

4. Conclusions

Equations based on log (M_l) = a + log (M_s) – pH + log (SOC) + log (DOC), where M_l and M_s represent metal concentrations in liquid and solid phases, respectively, described the relationship between metal concentrations in soil pore water and other soil properties satisfactorily for 72 sewage sludge amended soils. EDTA-extractable metals were more consistent than total metals in these empirical relationships. The positive influence of SOC on metal solubility may be a special feature in sludged soils, because SOC also reflects metal loading.

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HEAVY METAL LEACHING FROM SEWAGE SLUDGE-TREATED SOILS

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1. Introduction

In New Zealand, as in many other countries, there is increasing pressure for land treatment of sewage sludge. The accumulation of heavy metals in the soil is generally regarded as the major problem associated with this practice. As a result, guidelines or regulations are often set to restrict the accumulation of metals to levels that are unlikely to affect plant growth or soil biological activity, or lead to substantial transfer of metals into the food chain. It is generally assumed that since metals are strongly bound to the soil, leaching of metals downwards into groundwater is not an important issue. However, there are situations in which metal leaching from sludge-treated soils has been observed. For example, SIDLE and KARDOS (1977) and CAMERON et al. (1994) observed leaching of zinc (Zn) in sludge-treated forest soils. The objective of the current study was to examine the potential for metal leaching from a range of soils that had been amended with the maximum concentrations of sludge-derived metals permitted under the New Zealand Department of Health (1992) guidelines.

2. Materials and Methods

2.1 *Soils and Sampling Procedure.* Five contrasting soils were sampled for this study, 3 from forest (*pinus Radiata*) sites and 2 from pasture sites. Five undisturbed soil monolith lysimeters were taken from each site. Each lysimeter was 50 cm in diameter and 70 cm deep. The design of the lysimeter casings and the method of sampling the soils have been described in detail by CAMERON et al. (1992). The lysimeters were installed in field laboratories that enabled the collection of drainage leachate from the soils.
2.2 *Sludge preparation and application.* Sewage sludge was obtained from the Christchurch City Council treatment works and 'spiked' with additional metals (Cd, Cr, Cu, Ni, Pb and Zn) in the form of metal sulphates. The sludge was allowed to incubate with the metals for 6 months with occasional stirring before application to the lysimeters. Three replicate lysimeters of each soil were treated with sludge (3 L) to provide the approximate amounts of metals required to bring the top 10 cm of soil to the current NZ metal guideline limits (surface applied to forest soils, incorporated to 10 cm in pasture soils). Two lysimeters of each soil were left as controls. The lysimeters were exposed to natural rainfall and drainage leachate was collected over a period of 1 year.
2.3 *Leachate Analysis.* Leachates were filtered through a 0.45 µm filter and analysed for metals, major cations, inorganic anions, organic carbon and pH.

3. Results and Discussion

The 1997/8 year when this study was carried out was extremely dry in New Zealand (particularly the summer) and drainage volumes collected from the lysimeters varied from a high of 375 mm

to a low of 58 mm depending on soil texture. Leachate from the sludge-treated lysimeters generally showed far higher concentrations of Ca, Mg, K, Na, NO_3^-, Cl^- and SO_4^{2-} than leachate from the controls. Organic carbon was detected in most leachates but there were no consistent differences in concentration between sludge-treated and control lysimeters. Low concentrations of metals (Cd, Cr, Cu, Ni, Pb and Zn) were detected in most leachate samples from both the control and sludge-treated lysimeters. Although metal concentrations fluctuated substantially between samples and between the different soils, Cd concentrations were generally below 2 ng L^{-1}, Cr below 4 ng L^{-1}, Cu below 50 ng L^{-1} and Pb below 3 ng L^{-1}. Nickel and Zn showed a greater degree of variation between soils. For 4 soils, Ni concentrations were generally below 50 ng L^{-1}, but for 1 soil it was as high as 400 ng L^{-1}. Similarly for Zn, 1 soil produced leachate concentrations as high as 30 mg L^{-1}, whereas leachates from the other 4 soils were generally below 5 mg L^{-1}.

On 2 of the soils (a sand and a silt loam over gravel), sludge treatment resulted in substantially increased leachate concentrations of Ni and Zn and to a lesser extent Cd. On these soils, Zn leaching was increased by 1.1-4.2 kg ha^{-1}, Ni by 50-375 g ha^{-1} and Cd by 0.6-6 g ha^{-1}. However, this increased metal leaching represented very small proportions only (0.02-1.6%) of the metals applied to the soils in the sludge treatment. Sludge treatment had no effect on the leaching of Cr from the soils, but on 4 of the soils, sludge treatment actually decreased the leaching of Cu and Pb compared to the controls. This could be due to increased retention of these metals by the organic constituents of the sludge. Both Cu and Pb are known to form strong complexes with organic matter.

4. Conclusions

For some soils, application of sewage sludge can result in the increased leaching of certain metals (Cd, Ni and Zn) from the soil. However the concentrations of metals in drainage leachates are relatively low and, particularly where further 'diluted' by groundwater, would seem unlikely to cause significant environmental concerns.

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BIOAVAILABILITY OF CADMIUM AND ZINC 18 YEARS POST BIOSOLIDS APPLICATIONS

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Introduction

Post application bioavailability of biosolids-borne trace metals over extended periods of time is unresolved, a result of the lack of controlled, well-documented, long-term field studies. Berti and Jacobs (1996) reported continued phytotoxic concentrations of Zn in diagnostic maize (*Zea Mays* L.) tissue for 5 y following 10 y of biosolids applications. Bidwell and Dowdy (1987) reported that Cd and Zn uptake by maize decreased for 6 y post biosolids applications. Cumulative applications of Cd and Zn were 25 and 343 kg ha^{-1}, respectively. Utilizing soil from this field study, Sloan et al. (1997) reported elevated concentrations of Cd and Zn in romaine lettuce (*Lactuca sativa* L.) as a result of biosolids applications 16 y earlier. The objective of this paper is to report the bioavailability of Cd and Zn for maize uptake 18 y post biosolids applications.

2. Materials and Methods

A completely randomized block field study was established in 1976 with four replications and a plot size of 0.22 ha (Dowdy et al., 1983). Waste activated municipal biosolids were applied annually for 3 y for cumulative biosolids loadings of 0 (control), 60 (low), 120 (medium), and 180 (high) Mg ha^{-1}, a total Cd and Zn loading of 25 and 343 kg ha^{-1}, respectively, for the high treatment. The same maize hybrid was grown each year for the first 8 y and Year 16 and 18. Stover and grain were analyzed for Cd and Zn. Unless explicitly stated, discussions are restricted to Year 16 and 18 results.

3. Results and Discussion

Dry matter production was excellent. Biosolids applications had no significant effect on stover production ($P>0.05$) and seldom had any significant effect on grain yields. Hence, tissue composition was not confounded by differential biomass production. Biosolids derived Cd significantly ($P<0.005$) increased stover Cd concentrations (Table 1). There were no significant differences in stover Cd levels between biosolids treatments for Year 18; Cd concentrations were similar to those observed Year 3-6. The greater stover Cd observed during Year 16 (all treatments) was probably an anomaly based on Year 18 and Year 3-6 data, possibly confound by a preceding soybean crop (Oliver et al., 1993). Grain Cd concentrations were never enhanced by biosolids applications. Stover grown on treated soil accumulated more Zn than stover produced on the control treatment. By Year 3, stover Zn concentrations had stabilized with very few significant differences in Zn concentrations between biosolids rates. Unlike Cd, maize grain did accumulate biosolids Zn.

4. Conclusions

Biosolids applied Cd and Zn were still available for uptake by maize 18 y post application. Differences in stover Cd, and to a lesser extent Zn, concentrations were greater between the control *vs* biosolids treatments. Biosolids borne Cd did *not* accumulate in maize grain, while biosolids derived Zn did accumulate in the grain. Stover Cd and stover and grain Zn levels appeared to stabilize by Year 3 post biosolids applications.

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Table 1. Concentrations of Cd and Zn in maize post biosolids applications.

Tissue	Biosolids Applied	Year							
		1	2	3	4	5	6	16	18
	(Mg ha^{-1})	mg kg^{-1}							
		Cadmium							
Stover	0	0.23a++ 0.23a+	0.18a	0.11a	0.06a	0.08a	0.08a	0.24a	0.16a
	60	3.41b	1.48ab	0.82a	1.15b	1.82ab	0.88b	1.46ab	1.27ab
	120	4.96b	3.22b	1.72a	1.78bc	3.36b	1.26c	2.84bc	2.25ab
	180	9.83c	5.18c	4.56b	2.14c	3.26b	1.93d	4.63c	3.06b
	0	<0.06	<0.04	<0.02	<0.02	<0.02	<0.02	<0.03	<0.05
Grain									
	60	0.10a	0.05a	0.03a	<0.02	0.04a	0.04a	0.04a	<0.05
	120	0.11a	0.07a	0.07a	0.03a	0.04a	0.04a	0.07a	0.06a
	180	0.27a	0.06a	0.15b	0.05a	0.06a	0.06a	0.07a	0.07a
		Zinc							
Stover	0	19.9a	32.0	18.6a	30.8a	25.6a	18.4a	27.0a	15.1a
	60	88.9b	72.8ab	66.5b	88.0b	112b	66.6b	71.6b	50.6b
	120	101b	119bc	87.3bc	105b	114b	75.8bc	94.5b	84.6b
	180	140c	153c	121c	107b	154b	105c	125b	83.4b
Grain	0	25.6a	32.8a	27.6a	35.9a	36.7a	29.6a	25.4a	21.4a
	60	37.0b	41.0ab	39.3ab	43.4a	55.0b	37.3ab	29.5ab	25.3ab
	120	37.4b	42.1bc	46.8b	39.5a	55.4b	38.8b	32.5ab	27.6b
	180	44.7b	50.7c	72.2c	43.2a	55.3b	39.4b	36.3b	29.4b

+ Values within the same column and subset (e.g. Stover Cd) followed by the same letter were not significantly different at P=0.05.

THE BEHAVIOUR OF HEAVY METALS IN SLUDGE-AMENDED SOILS : APPLICATION TO LOW METAL LOADINGS.

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1. Introduction

The use of various waste-derived-amendments in agriculture is now of widespread practice. Sewage sludges disposal on arable land is considered as a method to utilize wastes and to improve soil fertility and soil conditions. However, the concentrations of several potentially harmful contaminants, such as heavy metals, commonly found in sludges, limit their application to land because of the risks for human health by accumulation in food crops. Generally, guidelines and legislation refer to the total amounts of heavy metals in wastes and in soils and recommend soil pH to be maintained at 6 or higher. Many studies on heavy metal uptake by plants grown on sludge-amended soils, have shown that several soil variables other than pH, such as texture, organic matter content, clay and cation exchange capacity, may influence the behaviour and availability to plants of heavy metals and that phytotoxicity symptoms may occur. Nevertheless, in most cases, phytotoxicity has been observed only in metal-sensitive crops grown on very strongly acid soils amended with high rates of sewage sludges. Due to the lack of knowledge of phytotoxic effects at low trace metal loadings, the aims of the present research are :

1. To evaluate the influence of the speciation of heavy metals from stabilized sewage sludges and their transformation after spreading onto three different soils, on their mobility and bioavailability.
2. To identify stress situations in plants, as indicated by changes in nutrients uptake and transport, and this for heavy metal contents in sludges below the recommended limit values.

2. Materials and Methods

A lysimeter experiment was carried out under greenhouse conditions. 58,5 kg of soil were filled into polyethylene pots and the first 10 cm of soil was mixed with sewage sludges at rates of 6, 10 and 30 $t.ha^{-1}$ (based on dried weight). The C horizon of an acid sandy soil (pH 5.6, 72% sand), the C horizon of an alkaline soil (pH 8.7) with 59% sand and 35% of silt and the Ap horizon of a silty clay soil (35% of clay and 54% of silt, pH 7.6), were amended with three composted sewage sludges. These sludges were anaerobically digested and dewatered by centrifuge and then composted with green wastes and bark. Each treatment was replicated twice. Controls were unamended. Pots were daily irrigated to keep the moisture content at 75% of the water holding capacity. Colza (*Brassica napus*, v. *Jaguar*) seeds were sown in each lysimeter at a rate equivalent to 5 $kg.ha^{-1}$.Soil samples were taken from each lysimeter at 0-10 cm depth (after sludge application and before sowing) and also at 10-20 and 20-30 cm depth (after harvest). The height of plants, the number and area of leaves were followed for each treatment during growth. Foliar samples were collected from each treatment at different periods of growth. Plants were harvested after 6 months of growth and immediately separated into roots, stems and leaves. Leachates from each treatment were collected before harvest. Soil and plant samples were dried, passed through a 0,1 mm sieve and subsequently analysed for their physico-chemical characteristics.For total trace metal concentrations, soil, sludge and plant samples were digested in *aqua regia*. The metal concentrations (Cu, Pb, Ni and Zn) were determined by furnace AAS, concentrations of Ca^{2+} and Mg^{2+} by flame AAS and concentrations of K^+ and Na^+ by flame AES. A chemical fractionation procedure modified from Tessier (1) was used to estimate exchangeable, carbonate, organic, hydrous oxide and residual forms of Cu, Pb, Ni and Zn in sludges, soils and sludge-amended soils.

3. Results and Discussion

First, in comparison with literature data (2), results indicate that composting induces a decrease of the representation of trace metals in exchangeable forms and therefore reduces their mobility and bioavailability. In all stabilized sludges, Cu and Ni are found mainly in the residual fraction while Pb is mostly organically bound and Zn is mainly extracted with the solubilized forms at pH 4.7. For all soils,

the application of composted sludges tends to increase the proportion of the solubilized forms at pH 4.7 of Zn and to increase the proportion of the organic forms of copper. The speciation of Pb in the amended soils differs with the type of soil. Composted sewage sludges increase substantially the organic matter, N and P contents of the soils. Soil pH values are very little affected by sludge applications.

Cu, initially associated with the organic fraction and mainly the residual fraction in all sludge-amended soils, do not display therefore, after one growing season, a great mobility into the silty clay soil and into the alkaline one (not farther than the 10-20 cm layer). However, the mobility of Cu increases as soil pH decreases. The mobility of Pb in the amended soils is not pH dependent. In comparison with the alkaline soil, Zn is more mobile in the acid and sandy soil and also in the silty clay one and reaches the 20-30 cm layer. Alkaline pH tends to favour metal retention and immobilization while acid media lead to solubilization of metal-humic material complexes (3). As the contents of metals in leachates are very low with respect to their concentrations in sludges, metal redistribution may occur in deep layers of these soils.Pb concentrations in colza grown on unamended soils (controls) and sludge-amended soils are below the detection limits ($<1 \mu g.g^{-1}$ d.w.). Cu and Zn bioavailability increases as soil pH decreases. The highest bioavailability of Zn is ascribed to its contents in sludges (higher than those of Cu and Pb) and to its representation in sludge-amended soils in exchangeable forms and mainly in easily solubilized forms at pH 4.7. No visual phytotoxic symptom were observed. Principal component analysis (PCA) was applied to plant data in order to study the uptake and transport interactions between nutrients and trace metals.

4. Conclusions

The pattern of metal distribution in the five fractions gives a good prediction of the metal behaviour in the sludge-amended soils. As a matter of fact, in contrast with zinc, lead and copper mainly extracted in the organic and residual fraction of the sludge-amended soils, do not present a great mobility and bioavailability. Potential risks could derive from decomposition of organic matter either due to mineralization or acidification processes which could lead, as a consequence, to the release of copper and lead in this experiment.

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Soil physical and chemical characteristics*

	Soil 1 (Ap horizon)	Soil 2 (C horizon)	Soil 3 (C horizon)
pH (H_2O)	7.6	5.6	8.7
Organic C, total ($g.kg^{-1}$)	20.2	9.2	8.9
N, total ($g.kg^{-1}$)	0.9	0.2	0.1
$CaCO_3$ total (%)	4.6	5.1	40.4
Available-P (%)	0.015	0.001	0.002
Exchangeable Na (meq/100 g of soil)	0.12	0.14	0.16
Exchangeable K (meq/100 g of soil)	1.07	0.03	0.1
Exchangeable Mg (meq/100 g of soil)	1.23	< 0.01	< 0.01
Exchangeable Ca (meq/100 g of soil)	2.16	0.04	145.5
Cu ($\mu g.g^{-1}$)	22.2	6	< 1
Pb ($\mu g.g^{-1}$)	25.4	27.6	4.8
Zn ($\mu g.g^{-1}$)	93	32.5	8.5
Sand (%)	11	72	59
Silt (%)	54	16	35
Clay (%)	35	12	6

* all data are expressed on a dry basis (60°C).

ASSESSMENT OF RISK FROM BIOSOLIDS-MO

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1. Introduction

Excessive availability of Mo in forage can induce a Cu-deficiency in ruminants, a condition known as molybdenosis. This possibility constituted the critical pathway in the USEPA's risk assessment for Mo in the land application of biosolids. Limited field data were available to adequately assess the risk, forcing EPA to make several simplifying assumptions and to use data that the Mo industry regarded as inappropriate. The resulting controversy and law-suit caused EPA to temporarily remove the Mo APL from Table 3 of 40CFR Part 503 until more suitable field data became available. Our three year field study of cattle grazing biosolids-amended pasture was designed to furnish such data. Incidentally, we also examined some of the fundamental assumptions inherent to this pathway's risk assessment.

2. Materials and Methods

Our test crop is bahiagrass, a pasture grass grown on >1 million ha in Florida, and a crop that readily responds to numerous biosolids-borne nutrients, e.g., N, P, S, Fe, Cu, and Zn. The dominate soil at our test site is the Millhopper fine sand, with a native pH of 5.6 to 5.9, SOM = 1 to 1.4%, and a low Cu-supplying capacity. Cattle grazing forage grown on this (and many FL soils) are routinely supplemented with Cu to avoid Cu deficiencies – a practice not followed in our study. Established pastures were amended with 3 "Exceptional Quality" biosolids, differing primarily in Mo contents (12 to 60 mg Mo/kg). Biosolids application rates routinely exceeded "agronomic" rates based on crop N needs to effect significant cumulative soil Mo loads, up to about 1.5 kg Mo/ha. Some treatments were applied only once and then studied as residual treatments, other treatments were applied in each of the three years. Thirty to 34 plots (depending upon the year) of 0.8 ha each were amended. Forage samples were collected monthly and analyzed for Cu, Mo, S, and a host of other nutrients and trace elements. Soil samples were collected twice yearly for similar nutrient and trace element analysis. Two (and later, 3) Angus yearlings grazed each plot for 5 to 6 months each year. Animals were sampled for plasma and liver tissue, which was analyzed for Cu and Mo. Animal condition, e.g., average daily gain, coat color, etc was also monitored.

3. Results and Discussion

Crop Mo concentration, expressed as seasonally (yield) weighted averages were related to soil Mo load (Figure 1) in an attempt to generate linear uptake coefficients (UC values) used in the EPA risk assessment algorithm. Plant Mo concentrations increased
with Mo load, but UC values from this (and a related, small plot) study varied linearly and non-linearly with soil Mo load, varied with soil pH, varied with biosolids source,
varied by month, etc. Animal tissues (plasma and liver) generally responded to treatments, and Cu-deficiency symptoms were noted, but forage and liver tissue Mo concentrations were never extreme. Low Cu concentrations in forage, plasma, and liver tissues were common, but generally poorly correlated with treatments. Biosolids treatments significantly increased forage S concentrations and, apparently, induced Cu deficiencies, aside from biosolids-Mo effects.

4. Conclusions

The current study is a realistic and thorough test of the Mo risk assessment that was the basis for the US sludge rule. Given the complexity of real world biosolids/soil/plant/animal systems, however, the risk assessment appears to have been too simplistic. For example, based on data collected here (and from other related studies) we conclude that:

1). Mo concentrations in forage, and UC values, are not simple linear functions of soil Mo loads, as was originally assumed,

2). Other soil, plant, or animal factors (not considered in the risk assessment) may be as important as Mo in causing (or protecting against) Cu-deficiencies, and

3). Normal animal management (e.g., mineral supplements, grazing rotation limits, etc.) and unknown factors may obviate Mo risk. Again, such factors were not considered in the rule.

Setting a meaningful, national Mo standard for biosolids will not be as straightforward as originally thought, and will likely require a new and more complicated algorithm. The new paradigm may need to consider site-specific data, management practices, and measurements of Mo form, speciation, availability, etc. not used in other metal risk assessments.

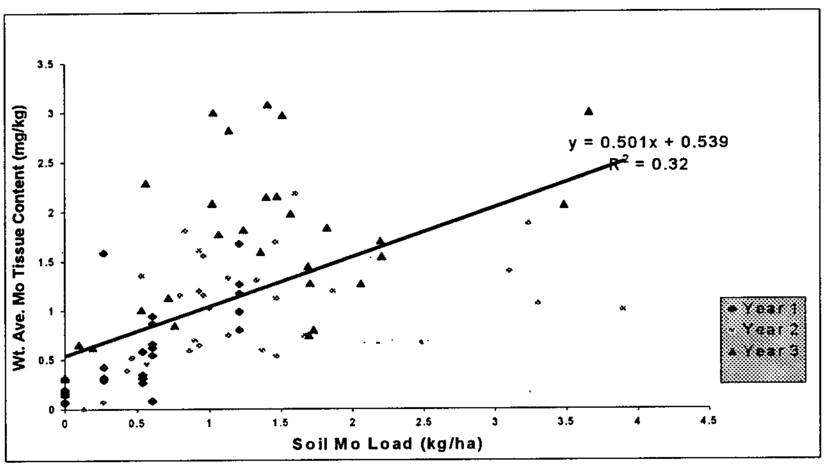

Figure 1. Linear model of plant-Mo with soil Mo load for combined Years 1, 2, and 3 data.

RELATIONSHIP BETWEEN BIOSOLID METAL CONCENTRATION, METAL FRACTIONATION AND BIOAVAILABILITY

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1. Introduction

There is currently conflicting evidence regarding the chemical forms of heavy metals in biosolids and their long term bioavailability to soil microorganisms and plants. Part of the current conflict may result from the fact that biosolids obtained from different treatment plants may demonstrate very different metal chemical forms and hence bioavailability (DeVries, 1981). We report the outcomes of a study that linked the behavior of metal chemical forms in two biosolids with very different initial metal fractionation profiles (determined using solid phase sequential extraction procedures), the behavior of biosolid metal fractions in soils, microbial ecotoxicological response and plant Cd uptake.

2. Materials and Methods

Biosolids were obtained form two treatment plants (MB and BO), and were applied to a sandy loam-mesic Molic Palexeralf, and Calcic-Rhodoxeralf, contained in concrete lysimeters. Biosolids were applied to the two soils at a rate of 500 t ha^{-1} equivalent. Soils were sampled 12, and 36 months after biosolid application. The distribution of metals between different fractions in biosolids and soils was operationally defined using the following extraction procedure: 0.1$\underline{M}$ Ca $(NO_3)_2$; 4.8% NaOCl pH 8; 0.2 $\underline{M}$ $C_2H_8N_2O_4$, 0.2$\underline{M}$ $C_2H_2O_4$, 0.1$\underline{M}$ $C_6H_8O_6$, pH 3.0; and *aqua-regia*. Metal microbial ecotoxicity was determined by biomass-C, microbial basal respiration, and qCO2 measurements. Plant metal bioavailability was determined by measuring wheat (*Triticum aestivum* var Jantz and Reeves) grain Cd at harvest.

3. Results and Discussion

The BO biosolid had higher total concentrations of Cd, and Zn, but the MB biosolid had a higher concentration of metals in the first two fractions extracted (Table 1).

Table 1 Cd and Zn concentration in sequential fractions from MB and BO biosolids

Extractant	Total Cd	$Ca(NO_3)_2$	NaOCl	Acid-oxalate	Aqua-regia	Total Zn	$Ca(NO_3)_2$	NaOCl	Acid-oxalate	Aqua-regia
	mg kg^{-1}									
MB biosolid	3.25	0.21	0.51	0	2.52	1609	76.14	66.86	1303.9	162
BO biosolid	13.6	0.14	0.21	0.18	13.06	2233	5.44	4.70	1937.6	285.13

The fractionation of metals resulting from biosolid application was almost identical in both soils studied, and at 12 months was similar to the metal fractionation in biosolids. No significant change in metal distribution in soils was observed at 36 months. Microbial biomass-C was reduced in the MB treatment (Fig 1), Biomass-C:OC ratios were lower and microbial metabolic quotient (qCO_2), had increased by 48% compared to the BO treatment. Grain Cd concentration was significantly higher in the MB treatment. Citrate-dithionate and oxalate extractable Al and Fe were measured in the two biosolids (Table 2). A much greater proportion of Al and Fe were extracted by the citrate-dithionate and oxalate extractants from the BO biosolid, indicating Al and Fe are present as amorphous forms (Parfitt and Childs 1988), with a high surface area and

potentially higher metal binding affinity, possibly explaining the lower bioavailability

Table 2. MB and BO biosolid Citrate-Dithionate and Oxalate extractable Al and Fe

			mg kg^{-1}			
Biosolid	Total Al	Citrate-Dithionate Al	Oxalate extractable Al	Total Fe	Citrate-Dithionate Fe	Oxalate extractable Fe
MB	15086	2600	2500	18026	9300	7000
BO	26105	20650	17600	13824	11000	9300

4. Conclusions

This study has established that the chemical forms of metals in biosolids from different treatment works is not consistent, and that there is a strong relationship between concentrations of metals in the readily extractable forms and bioavailability to both plants and microorganisms.

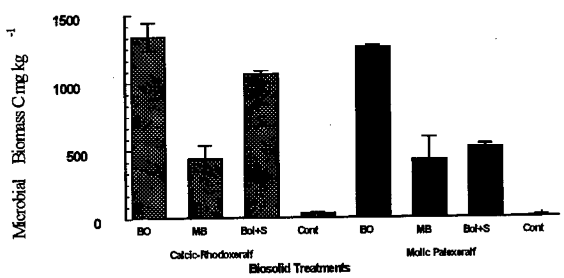

Figure 1. Microbial Biomass C in soils receiving MB and BO Biosolids

5. References

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EFFECT OF FE OXIDES ON THE BIOAVAILABILITY OF TRACE METALS IN BIOSOLIDS

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1. Introduction

Organic material (OM) in biosolids, when applied to the soil, binds a large proportion of trace metals. However as the OM content decreases with time, the metals may become more bioavailable. Common inorganic soil components with significant binding ability for the trace metals are Fe oxides. Studies have shown that Fe oxides have a binding preference for specific metals (Rule, 1998; Yong et al., 1992) which becomes important when high levels of several metals are applied to soils. With high concentrations of metals added, the capacity of specific sorption sites (strongly bound metals) could be exceeded and the metal cations would compete with the bulk cations for adsorption sites.

Biosolids may contain a significant proportion of Fe oxides. Many Publicly Owned Treatment Works use a flocculation and P removal process that produces $Fe(OH)_3$. When such biosolids are land-applied, Fe oxide formed in the process might increase long-term stability of trace metals. Alternately, Fe oxide could be added directly to soils that have received applications of biosolids to supplement naturally occurring oxides with more reactive forms. Addition of Fe oxides would be particularly appropriate for sandy textured soils.

2. Materials and Methods

Biosolids were air dried, ground to <2mm and homogenized. Samples were treated with a solution containing 100 mg L^{-1} of each Cd, Cu, Pb and Zn as nitrates. Another set of samples was treated in the same manner but with the addition of an Fe oxide product (5% by wt), Fe-Rich™ (E.I. Du Pont de Nemours, Wilmington, DE). After equilibration for 15 hrs on an end-to-end shaker, the samples were centrifuged and the metals remaining in solution determined by AAS. There were three replicates of each treatment. The solid samples were then subjected to a sequential selective extraction (Rule, 1998) for the operationally defined phases: Water Soluble (WS), Exchangeable (EP), Carbonate (CP), Easily Reducible (ERP), Moderately Reducible (MRP), Organic-Sulfide (OSP) and Acid Extractable (AEP). Metals in the extracts were determined by AAS.

3. Results and Discussion

The untreated biosolids and biosolids + Fe-Rich™ adsorbed Cd, Cu and Pb from the spiking solution but released Zn into the solution (Table 1). The desorption of Zn was due to repartitioning caused by the competitive adsorption of Cd, Cu and Pb, which followed reported sorption order preference (Rule, 1998). Biosolids treated with Fe-Rich™ sorbed significantly more of all metals with the exception of Pb compared with the untreated biosolids.

Addition of the Fe-Rich™ increased Cd and Zn concentrations in the MRP fraction and increased levels of all metals in the AEP, although only slightly for Zn. The dramatic increase in metal concentrations in the AEP with the addition of Fe-Rich™ indicates that a portion of the oxides present is chemically resistant to the MRP reagent (Fig1). The MRP reagent targets amorphous Fe-oxides to extract the most soluble and reactive of these oxides. The proportions of the metals sorbed to the Fe- Rich™ followed the order Pb>>Cu>Cd>Zn. The high proportion of Pb in the AEP of the biosolids might have been due to sorption onto very resistant humus but most likely was due to binding onto the inorganic fraction of the biosolids material.

4. Conclusions

Several studies have determined that metals in the Fe oxide and acid extractable phases are not bioavailable and are geochemically stable. The presence of these chemically resistant Fe oxides should result in long term stability of trace metals in soils due to the oxide's ability to bind the metals long after organic material is gone.

After the addition of Fe-Rich™, a greater proportion of all metals, including Pb, was present in the AEP, the most resistant soil phase indicating a reduced bioavailability of all four metals. This research has demonstrated the feasibility of addition of a Fe oxide product to biosolids for the purpose of decreasing bioavailability and increasing long-term immobility.

5. References

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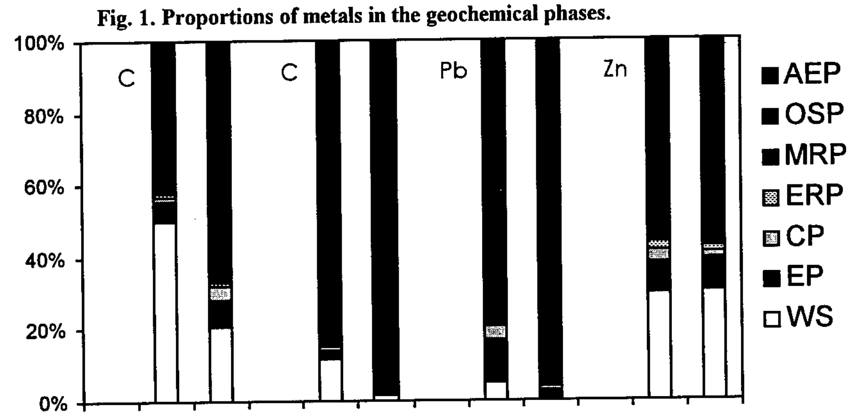
Table 1. Concentrations of metals in solids before and after spiking.

				Sum of Extr'ns after spiking	
Element	Fe-Rich™	Biosolids	Biosolids + 5% Fe-Rich™	Biosolids	Biosolids + 5%Fe- Rich™
mg kg^{-1}					
Cd	71	12	15	211	342
Cu	2404	648	762	691	926
Pb	1012	207	255	659	524
Zn	5748	2258	2532	727	987

Fig. 1. Proportions of metals in the geochemical phases.



BS=Biosolids
BS+Fe=Biosolids+Fe-Rich

Solids Utilized

MANAGEMENT OF SEWAGE-SLUDGE AMENDED SOILS TO ALTER HEAVY METAL "BIOAVAILABILITY"

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1. Introduction

Disposal of wastewater treatment sludge onto agricultural land in New Zealand has led to the development of guidelines to minimise the impacts of contaminants on the environment and the human food chain (e.g., NZ DoH, 1992; NSWEPA, 1995). These regulations prescribe upper limits for heavy metal concentrations in the sludge and, generally, maximum single and cumulative additions to the land. They also give upper limit concentrations for metals in the underlying soil, based on *total* metals present, affording little recognition of soil type or land management practices. The total concentration includes heavy metals derived from natural geochemical processes and does not take into account that these may have very different bioavailability and bioaccumulation properties from their sludge-borne counterparts.

In this paper, we provide data on heavy metal concentration in soil solution from soils under pasture treated with undigested sewage sludge from the New Plymouth Wastewater Treatment Plant, New Zealand. The sludge from this facility, which serves a permanent population of 44,000, contains moderate levels of Cr, Cu, Ni and Zn, mainly derived from industrial sources.

2. Materials and Methods

The sludge application area comprises 8 ha of low fertility land on coastal dune sands. In total, 9300 tonnes sewage sludge were applied between May 1991 and August 1994. The sludge was surface applied and the soil tilled to mix it through the top 20 cm; this was repeated 6 months later. The treated area was then oversown with ryegrass/clover and used for cattle grazing.

In March 1995, we dug pits in each of five paddocks (Blocks) which had received sludge at different, but often overlapping, times, and sampled at 0-5, 5-10, 10-20, 20-30, 30-50 and 50-80 cm depth for analysis of total and soil solution metals. Soil solution was extracted by dense liquid displacement. Total metals were analysed by x-ray fluorescence spectrometry and solution metals by inductively-coupled mass spectrometry. Concerns raised about very low soil pHs in the treated blocks and the absence of clover on the site, prompted the landowner to begin a liming programme which continued over the next two years. We repeated our sampling and analysis programme in March 1996 and March 1997. GEOCHEM-PC was used to determine the free-metal ion (major "bioavailable" fraction) concentration in the soil solutions.

3. Results and Discussion

Total soil concentrations of Cr, Cu, Ni and Zn in the 0-5 cm sample from the treated plots ranged from 90-200, 50-250, 21-58, and 220-440 mg/kg, respectively. Concentrations generally remained relatively constant in the top three samples (0-5, 5-10,10-20 cm depth) then declined with depth, although the decline was often not as great as expected. The untreated control soil had high concentrations of all metals, especially Ni (16-41 mg/kg) and Zn (210-280 mg/kg), which may have accounted for the less than expected decline with depth in the treated plots.

Soil solution Cu, Ni and, especially, Zn free-ion concentrations were very high in the sludge-treated blocks in 1995, when soil and soil solution pHs were low. The highest concentrations were found in the 5-10 and 10-20 cm samples, which had the lowest pH values. The results for Zn in Blocks 1 and 3, compared with the untreated Control Block, are shown in Fig 1. After liming, soil pH values increased in 1996, but in some instances declined again in 1997. Soil solution metal concentrations were, however, much lower at these two sampling times (e.g., Zn in Fig 1).

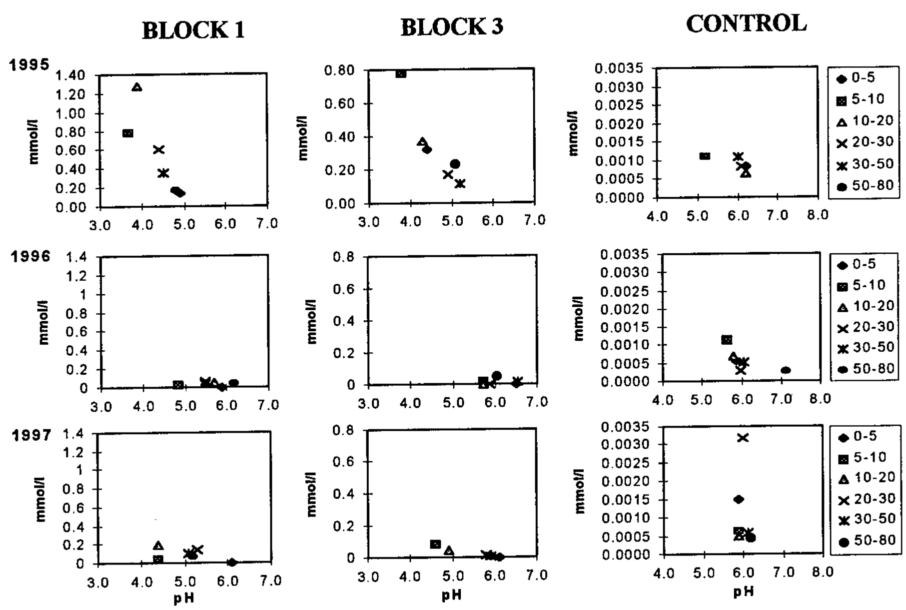

Fig 1. Soil solution Zn free-ion concentrations as a function of soil pH

Note the very large difference in scale between the sludge-treated blocks and the untreated Control Block.

Two factors emerge from these results. Firstly, liming the soils decreases the concentration, and presumably, the bioavailability of metals in soil solution. This is not totally a pH effect, as can be seen for Zn in the 1997 samples where the pH values have declined again but solution concentrations have not increased markedly. Secondly, even though the Control soil has naturally a relatively high Zn concentration, the amount in soil solution is very low. This shows that the native soil Zn is far less bioavailable than the sludge-borne metal.

4. References

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APPLYING BIOSOLIDS TO ACID SOILS IN NSW, AUSTRALIA: SUSTAINED AVAILABILITY OF CD 8 YEARS AFTER APPLICATION

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1. Introduction

The long-term reactions of biosolids-metals with soils of pH_C <5.0 have not been widely reported. Such soils are common in many areas of coastal NSW and are also proximal to the main centres where biosolids originate. Amending the pH of these soils is often not a long-term, or economically viable, management option.

2. Materials and Methods

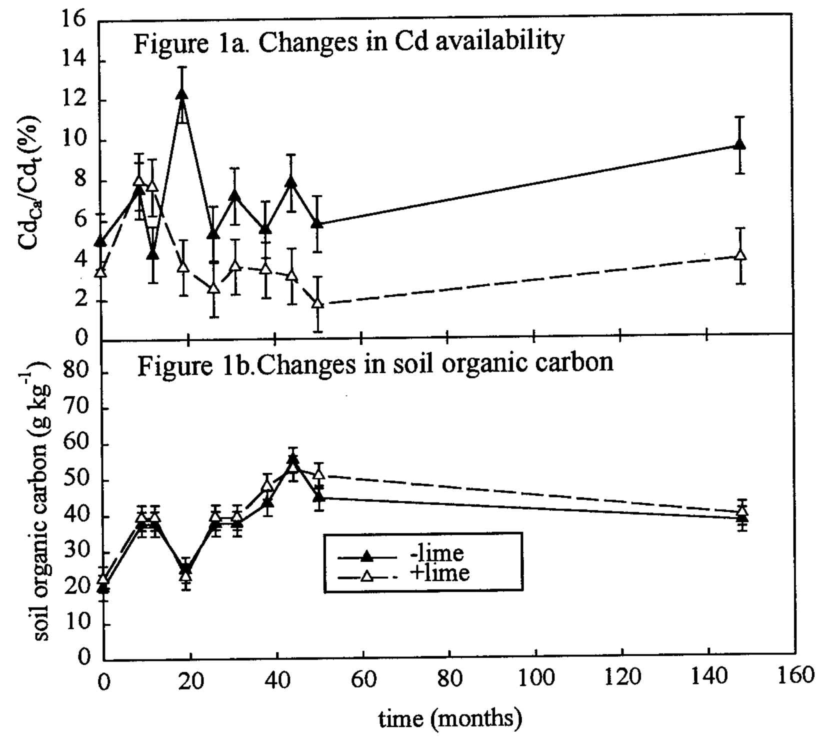
The trial consists of 24 plots (5 x 50 m) with three replicates and is situated on a Glossaquic Paleudalf, 70 km SW of Sydney. Topsoil (0-10 cm) pH_C (in 0.01*M* $CaCl_2$) is 4.4-4.8 and organic carbon (OC) levels are less than 20 g kg^{-1}. Between 1983 and 1987, low-metal, liquid biosolids (Cd 1.8-7.2 mg kg^{-1}, Cu 700-2,800 mg kg^{-1} and Zn 400-1,650 mg kg^{-1}) were added to the soil surface annually at rates of 0, 40 and 120 dry t ha^{-1}, +/- lime (2 x 2.5 t ha^{-1} lime). Soil Cd concentrations reached 2.2 mg kg^{-1} after 8 applications. In 1993, a second trial (72 plots 1.5 x 2 m) with 3 replicates was established overlying part of the original site. To assess biosolids metal reactions under extreme conditions, a one-off application of high-metal, dewatered biosolids (Cd 65 mg kg^{-1} and Zn 2,600 mg kg^{-1}), was applied at 0, 12, 50 and 100 dry t ha^{-1}. Soil pH was amended to pH_C 4.2, 4.8 and 5.8.

3. Results and Discussion

During the first trial, total soil Cd concentrations increased with each biosolids application, and have remained constant since biosolids application ceased in 1987. Often it has been shown that where high-metal biosolids are applied, the metals may become more readily available for plant uptake, than where low metal biosolids are used. It was therefore thought that the metal uptake response by plants grown on our soils amended with the high-metal biosolids would overestimate uptake where the low-metal material only was applied. To assess this, plant uptake models derived from the one-off, high-metal biosolids treatments, were used to predict uptake from soils receiving only low-metal biosolids. These were then compared to actual metal concentrations found in plants grown on these treatments, 7-8 years after the low-metal biosolids were applied. Contrary to expectation, at the lower soil pH, the models derived from high-metal biosolids did not overestimate measured Cd concentrations in plants grown on soils amended with the low-metal biosolids. Changes over time in the proportion of total soil Cd potentially available to plants are presented in Figure 1a. These data are for soils amended with the low-metal biosolids, applied at the lower rate (40 dry t ha^{-1} yr^{-1}). In the 8 years since these biosolids were last applied, the level of Cd potentially available for plant uptake has remained constant or has increased, especially on the low pH treatment. Similar trends were also seen for the high rate treatment, and for Ni and Zn. During this same time period soil organic carbon has decreased by up to 20 % (Figure 1b).

4. Conclusions

Under these acid soil pH conditions (pH_C<5.0), some of the biosolids-Cd has remained highly plant-available, despite the low levels found in the biosolids. Plant uptake models derived from high-metal biosolids under the same pH conditions did not overestimate uptake when low-Cd biosolids were used, possibly because the acid soil pH prevents immobilisation of the plant available Cd by either inorganic or organic components in the soil or biosolids.



BIOAVAILABILITY OF TRACE METALS IN CROPS AS INFLUENCED BY RADIATION PROCESSED SEWAGE SLUDGE

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1. Introduction

In recent years there has been a concern regarding the utilization of sewage sludge on agricultural land. Its use is reported to increase crop production because it contains some essential plant nutrients (Hermandez, et al., 1991). Sewage sludge also contains trace elements in high concentrations which can adversely affect human and animal health through the food-chain. A study was, therefore undertaken to assess the bioavailability of trace metals to crops as influenced by non-irradiated and radiation processed sewage sludge.

2. Materials and Methods

Sewage sludge collected from a sewerage treatment plant was air dried and processed for irradiation by Co--60 gamma ray at a dose rate of 5 kGy. Some physico-chemical characteristics of both radiation processed and non-irradiated sewage sludge along with the experimental soil were determined.

A field experiment was carried out in RCBD on a dark grey floodplain soil (Haplaquepts) having a sandy loam texture. The experiment consisted of 10 treatments such as T_1 –(-100 kg N ha^{-1}) from urea, T_2(-20 kg N ha^{-1} from urea T_3(-100 kg N equiv.), T_4(-200 kg N equiv.), T_5(-300 kg N equiv.), and T_6(-400 kg N equiv. ha) from non-irradiated sewage sludge, T_7(-100 kg N equiv.), T_8-(200 kg N equip.), T_9(-300 kg N equiv.) and T_{10}(-400 kg N equiv. ha^{-1}) from radiation processed sewage sludge.

3. Results and Discussion

Some physico-chemical properties of the sewage sludge and the experimental soil are presented in Table 1, showing relatively trace metal contents as recorded from aqua-regia extractant. Significantly higher wheat grain yields of 2.6 and 2.8 t ha^{-1} were recorded from non-irradiated and radiation - processed sewage sludge, respectively, both applied at the rate of 400% N equivalent ha^{-1}.

Trace metal concentrations in grain and straw of wheat are presented Table 2. Zn content was, in general, higher in both grain and straw. The concentration of Cu in grain was comparatively lower. Pb and Ni concentrations were considerably low. The Cd content in wheat grain was lowest and ranged between 0.02-0.03 mg kg^{-1} and in some cases remained below the detectable limit. The data obtained on trace metal contents in plants showed no significant difference between non-irradiated and radiation - processed sludge treatments. Trace metal losses from soil by crop removal and leaching are generally small as reported by Chang et al., (1984) and Dowdy et al., (1991).

4. Conclusions

It may be concluded from the present findings that trace metal concentrations in crop remained within the optimum range thus indicating no toxicity in crop from the application of sewage sludge. The trace metals are probably less bioavailable and as such there was little removal by crop. Therefore, sewage sludge can be used as an organic amendment to improve soil fertility and crop yield.

5. References

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Table 1. Some physico-chemical characteristics of sewage sludge and initial soil under study

Parameter	Non-irradiated Sewage sludge	Radiation processed sewage sludge	Experimental soil
PH (1:5 sludge $CaCl_2$)	5.3	5.2	6.2
Org. Carbon (%)	14.5	14.0	0.89
		Aqua-Regia Extract	
Zn (mg kg^{-1})	688.5	684.8	50.0
Cu (mg kg^{-1})	256.0	252.0	27.6
Cd (mg kg^{-1})	4.5	3.4	*
		DTPA Extract	
Zn (mg kg^{-1})	120.0	116.9	10.2
Cu (mg kg^{-1})	14.5	13.2	8.0
Cd (mg kg^{-1})	0.85	0.92	*

• Below detectable limit

Table 2. Trace metal concentrations in grain and straw of wheat

Treatment	Grain					Straw				
	Zn	Cu	Cd			Zn	Cu	Cd		
	mg kg^{-1}					mg kg^{-1}				
T_1	35.2	3.67	*			30.5	6.2	*		
T_2	34.0	4.17	*			30.9	7.0	*		
T_3	50.6	3.67	.03			31.6	7.0	0.2		
T_4	50.6	4.00	.02			36.1	6.2	0.2		
T_5	51.5	4.00	*			45.5	6.5	*		
T_6	47.6	4.33	.03			54.5	7.0	0.4		
T_7	45.5	4.33	*			43.0	6.3	*		
T_8	49.1	3.67	.02			44.10	6.5	0.3		
T_9	47.9	4.00	.02			52.7	6.5	0.2		
T_{10}	51.5	4.67	.03			56.9	7.0	0.3		
LSD(P=0.05	3.2	NS	-			6.8	NS	-		

* Below detectable limit

THE DISTRIBUTION OF HEAVY METALS IN PLANTS GROWING IN SOILS TREATED BY SEWAGE SLUDGE

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1. Introduction

Sewage sludge contains on one side organic matter and wide range of nutrients, on the other side organic pollutants and potentially toxic elements. Among them Cd, Zn, and Hg show serious troubles. The effect of sewage sludge application in three soils on Cd, Zn, and Hg uptake by three crops was investigated.

2. Materials and Methods

Sewage sludge with the total content of 1524 mg Zn, 4.67 mg Cd, and 4.85 mg Hg per kg of dry matter was tested at three soils: Chernozems (location Suchdol), Luvisols (l. C. Ujezd) and Fluvisols (l. Prerov). Soils from unpolluted arable land different in organic matter, soil pH, and CEC were chosen for the experiment. Fresh sewage sludge in the rate of 20 t of dry matter per ha was thoroughly mixed with 5 kg of air dried soil and with ammonium nitrate. Three crops were planted at each soil: barley (var. Akcent), wheat (var. Saxana) and maize (var. hybrid DK 205). Grains were harvested at full maturity and maize at the height of 100 cm. Concentration of elements was determined by atomic absorption (Cd and Zn by Varian SpectrAA-40 and Hg by AMA-254). Accuracy of analyses was tested by certified materials.

3. Results and Discussion

The application of sewage sludge led to a yield increase of growing crops. The highest increase of the yield by 56.2 % was found at Luvisols, lower by 10.3 % at Chernozems and by 6.3 % at Fluvisols. Relative changes of contents of heavy metals (mean of all above ground analyses) are shown in Fig. 1 (I-III). The sludge application showed the significant effect on the Cd and Zn accumulation in above ground biomass. Cd content in biomass was higher by 50 % at Chernozems, by 28 % at Fluvisols and only by 3 % at Luvisols. Zn biomass content was higher by 37 %, 45 %, and 24 % at the same treated soil. Lower Hg content was found at Chernozems and Luvisols due to low uptake by plants and dilution yield effect of plants at treated soils. Roots showed higher differences of all metals content than above ground biomass (IV-VI). Hg was the highest accumulated in roots.

4. Conclusions

The application of high amount of sewage sludge led to significant increase of Cd and Zn content in above ground biomass and in roots of all plants. Higher Hg content was mostly found in roots. The element mobility in plants decreased in order Cd > Zn > Hg.

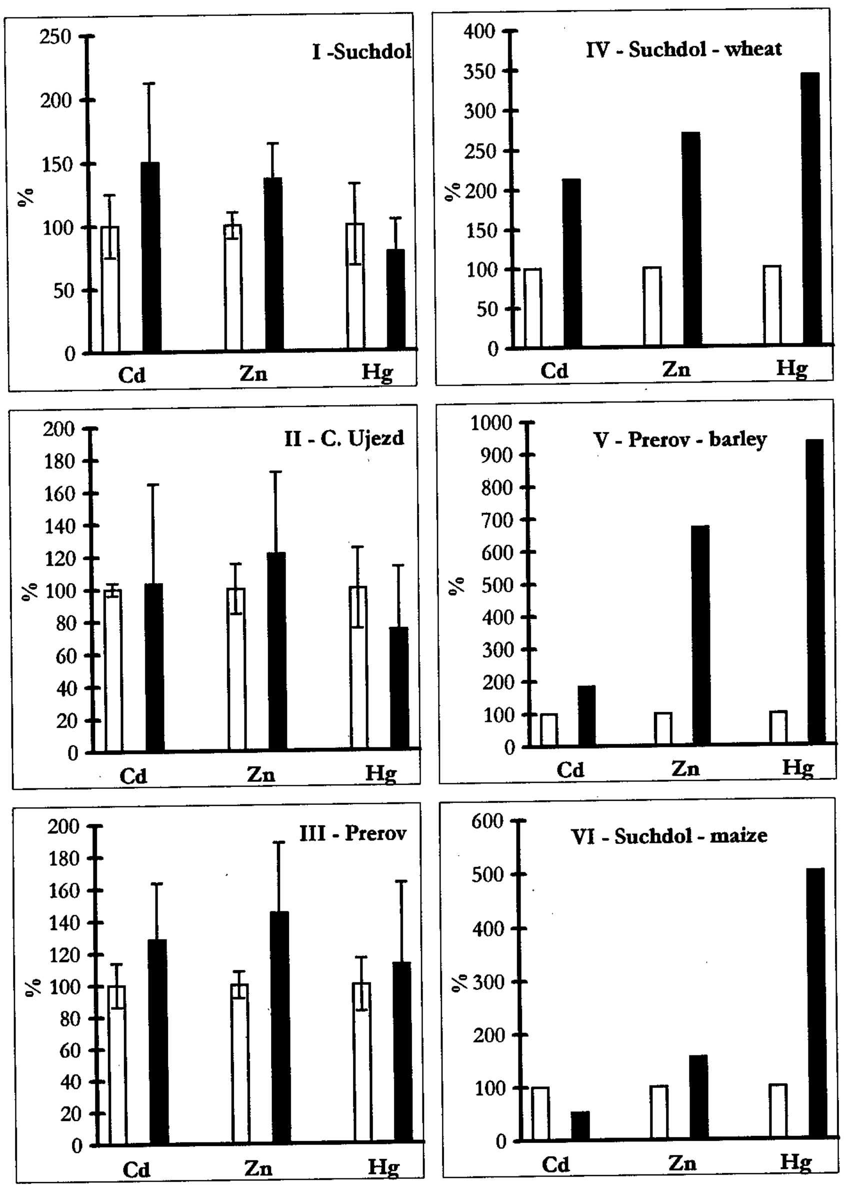

Fig. 1: The relative changes of heavy metals concentration in above ground biomass (I - III) and in roots of selected crops (IV - VI) in three soils treated by sewage sludge (untreated; ■ sewage sludge)

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METAL ION ACTIVITIES IN LONG-TERM BIOSOLID-AMENDED SOIL

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1. Introduction.

Long term application of sewage sludge (SS) to cropland may result in an accumulation of heavy metals such as Zn, Cu, Ni and Cd in the soil, which can enter the food chain after being absorbed by plants. Some farm managements like N fertilization with urea or ammonium sulfate can further increase heavy metal bioavailability by their effect on soil acidity. Since metal uptake by a plant is largely a function of its activity in soil solution, an experiment was carried out to determine the effect of changes in metal ion activities in samples of a sludge-amended soil of varying pH.

2. Materials and Methods.

Soil samples were taken, in 1994, from a long term SS land application experiment established in 1976 on a Ramona sandy loam soil (fine-loamy, mixed, thermic Typic Haploxeralf). The composition of the SS compost used is as follows: major elements in %: Ca: 17, Mg: 1.4, Na: 0.6, Al: 0.6, Fe: >1.6, K: >0.4, P: 1.0; minor elements in mg kg^{-1}: B: 50, Cd: 40, Cr: 600, Cu: 475, Ni: 250, Pb: 1100, Zn: 3547, V: 35. From 1976 through 1981 composted sludge was applied at dry weight rates of 0 (control) , 22.5, 45, 90 and 180 Mg ha^{-1} yr^{-1} on the experimental plots. The annual SS mass loading was divided into two equal application and applied in spring and fall of each year. Vegetables were planted and harvested following each application. At the end of this experiment, plots had received a total of 0 (control), 135, 270, 540 and 1080 Mg ha^{-1} yr^{-1} of sludge compost. Changes in metal ion activities were measured in a two-chamber membrane diffusion apparatus, which consisted of two chambers separated by a polycarbonate membrane with 0.4 μm pores. Two grams of the sludge-amended soil samples were equilibrated overnight with 250 mL of a background electrolyte solution (BES), and placed in one chamber. The other chamber was filled with BES or BES plus 97.8 μmols of H^+ for rate 0 (control) and 56.4 μmols of H^+ for rates 22.5, 45 and 180 Mg ha^{-1} yr^{-1}, from a 0.1M HNO_3 solution. After the system had reached steady state (5 days shaking) solution phase samples from the chamber that received only BES with or without HNO_3 addition were collected and analyzed for cations (ICP-ES), anions (Dionex Ion Chromatograph), total organic carbon (Dohrman DC-80 Carbon Analyser) and pH. This data was then input into the GEOCHEM-PC program to determine the speciation of Zn, Cd, Ni and Mn in solution , according to PARKER et al. (1995)

3. Results and Discussion.

Activities of Zn, Cd, and Ni increased quadratically with the amounts of SS applied, while it had no effect on Mn activity (Table1). Addition of H^+ as HNO_3 decreased the solution pH to 6.2 ±0.1, and, within each rate applied, increased the activity of all metals ions in solution, except for Cd at rate 0 (control). Activities of Zn, Mn and Ni were increased as a result of H^+ addition to the soil that received no SS amendment, and appeared to decrease as the amount of SS applied to the soil increased. This suggests that a component present in the SS, probably organic matter, is preventing the metal from being released to the solution as pH decreased. Considering only the sludge amended treatments, Cd was the metal that had the greatest activity increase as the

solution pH decreased, and was followed by Zn. Cd activity at the rate of 22.5 Mg ha^{-1} yr^{-1} increased 1.6 fold as solution pH decreased from 6.9 to 6.3.

Despite the increase in metal ionic activity as pH decreased, the percentage of the free species of the metals present in solution also increased, with Zn showing the greatest difference, about 8.5% on average, and less than 3% for the other metals under investigation.

Table 1.Change in metal ion activities (x 10^{-6}) and in their free percentage in soil solution in relation to pH in a long-term sludge-amended soil.

		Zn^{+2}		Mn^{+2}		Cd^{+2}		Ni^{+2}	
SS* Rate	solution pH	activity	% free	activity	% free	activity	%free	activity	% free
Mg ha^{-1} yr^{-1}									
0	7.1	0.07	71	0.86	81	0.008	53	0.03	81
	6.1	0.58	81	1.68	82	0.008	54	0.15	83
22.5	6.9	0.86	74	0.87	80	0.008	52	0.17	80
	6.3	1.73	81	1.43	82	0.021	54	0.30	83
45	6.8	1.05	76	1.05	81	0.010	53	0.32	81
	6.2	2.69	81	1.77	82	0.027	55	0.48	83
180	6.6	1.83	77	1.04	81	0.013	54	0.56	81
	6.1	3.30	82	1.67	82	0.030	55	0.75	83

SS* = Sewage sludge

4. Conclusions

Except for Cd at rate 0 (control), the activities of all metals increased by decreasing the solution pH. There was an exponential increase for Zn, Cd and Ni and it was higher for Cd and Zn on the SS amended treatments.

The percentage of the free metals present in solution increased mainly for Zn (8.5%) and less for Cd, Ni and Mn (3%).

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TRACE METALS CONTENT IN SOIL AFTER LONG TERM APPLICATION OF SEWAGE SLUDGE AS A FERTILIZER.

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1. Introduction

The use of sewage sludge as a fertilizer requires due thought and attention. It is important that the soil is not polluted with unwanted trace metals which have a prolonged negative influence on the quality of crops.

Application of sludge can cause an increase in the soil trace metal content and modify their plant availability.

The aim of this paper is to study the long term acccumulation of trace metals in soils from a field experiment with continuing application of urban sewage sludge.

2. Materials and Methods

Experimental plots : Comparaisons were made between two types of nitrogen fertization for a maize crop : one with urban sewage sludge and one with mineral fertilizer. The various treatements consisted of four replicates.

The plots were alternatively cultivated with maize and corn. Sewage sludges were applied every two years (before maize culture). The rate of sludge application was 6 tonnes dry matter per ha. The study was set up in 1985.

The experimental soil is a deep loam soil (clay 21%, silt 74%, sand 5% ; pH 6.6 ; organic matter 2.3% ;CEC 120 cmol kg^{-1} .

Sewage sludges : the sludges were urban liquid anaerobic stabilized sludges with a relatively low content of trace elements (less than the limit values for allowable metal content in sludge when use on arable soil in France, except for chromium and nickel). The contents of sludge trace metals over the years 1985-91 are presented in table 1.

TABLE 1: Composition of sludge, 1985-91 (/ Dry matter : MS)

Year		1985	1987	1989	1991
% Dry matter (M.S.)		3.7	3.8	5.4	2.2
% sur M.S. Organic C (ANNE)		29.1	31.2	31.4	31.9
Organic matter		50.1	53.6	54.0	54.9
Total N (KJELDAHL)		5.5	5.6	4.5	4.8
N NH4		1.6	1.8	1.7	3.2
Total P (HF)		2.4	3.0	2.8	2.2
	Limit values*				
Mg/kg de M.S. Total Copper	1000	450	478	545	533
Total Zinc	3000	1027	855	852	872
Total	1000	1170	735	1790	938
Chromium	200	320	295	105	73
Total Nickel	800	157	124	113	95
Total Lead	20	4.2	2.1	3.1	6
Total Cadmium	10	2.8	6.5	2.8	4.6
Total Mercury					

* limit values for allowable metal content in sludge when used on arable soil in France

Studied parameters : After the 8 experimental year, soil samples were taken at 30 cm intervals to a depth of 90 cm. Soil sample were analysed for trace metals (Cu, Zn,Cr, Ni, Pb, Cd, Hg). The chemical analyses of the crops were performed every year for all the plots of the experiment.

3. Results and discussion

The total quantities of trace elements supplied by the sewage sludges is relatively weak except for chromium, zinc and nickel (Table 2).These experimental values may be compared with the calculated increase of the trace elements content of the top soil (0-30 cm) assuming a quantitative fixation of trace elements at the soil surface.

TABLE 2 : Amounts of trace metals applied by four applications the sludge (1985-93)

Metal	Cu	Zn	Cr	Ni	Pb	Cd	Hg	M.S. (t/ha)
Amount (g/ha)	12120	21650	30065	4835	2990	85	95	24
Calculated increase top soil (mg/kg)	+2.7	+4;8	+6.7	+1.1	+0.7	+0.02	+0.02	

Soil analyses in the top 30 cm show that no significative increase of the trace metal contents was due to the sewage sludge fertilizer after the 8 experimental years. (Table 3).

TABLE 3 : Content of trace metals in the top 30 cm of the soil after the 8 experimental years

	CONTROL (T) ppm					SLUDGE FERTILIZER (S) ppm					
	B I	B II	B III	B IV	[1]*Mean T*	B I	B II	B III	B IV	[1]*Mean S*	**(S) - (T)**
Cu	22.5	23.8	18.6	22.1	**21.8±2.2 a**	31.6	24.8	32.0	32.7	***30.3±3.7 h***[2]	**8.5**
Zn	74.0	89.0	70.0	79.0	***78.0±8.2 b***	85.0	80.0	80.0	83.0	***82.0±2.4 b***	**4**
Cr	59.0	54.6	46.3	52.9	***53.2±5.2 c***	58.3	56.2	63.0	59.9	***59.4±2.8 c***	**6.2**
Ni	21.3	24.3	20.2	22.6	***22.1±1.8 d***	23.0	20.5	19.8	21.7	***21.3±1.4 d***	**-0.8**
Pb	38.8	39.1	40.6	45.0	***40.9±2.9 e***	44.2	44.0	46.4	46.1	***45.2±1.2 e***	**4.3**
Cd	0.21	0.27	0.23	0.25	***.24±0.02 f***	0.24	0.24	0.25	0.24	***0.24±0.005 f***	**0**
Hg	0.12	0.16	0.14	0.17	***.15±0.02 g***	0.16	0.16	0.19	0.19	***0.18±0.015 g***	**0,03**

[1] *Mean Values ± Confidence Interval $p \leq 0{,}05$*

[2] *Values with the same letter in a line are not significantly different ($p \leq 0{,}05$ Newmann-Keuls test)*

The comparison between calculated increase and the content metal differences (means; table 3) in soil with sludge and those without control indicated only for zinc and chromium the same order of magnitude. The content of metals in the underlying layers remained the same as before sludge application.
For crops, no significant increase of the trace metal content was observed.

4. Conclusion

Long-term-field experiments are necessary to assess the interest of the agricutural usage of urban sewage sludges as a fertilizer and to anticipate the long-term effects of their application on the risk of trace metal accumulation in soils and crops. The results obtained after the 8 experimental years indicate that no significant effect on plants and soils of trace metal content of the sludges may be observed

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HEAVY METAL IN SOIL AMENDED WITH MUNICIPAL SOLID WASTE COMPOST FOR TEN YEARS.

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1. Introduction

Soils cultivated with vegetable and fruits (guava, coconut, banana, lemons) near Rio de Janeiro town, require high levels of organic amendments to their productivity. Municipal solid waste compost (MSW) is an accessible source of organic input to this agricultural activity. Considering the world research and analysis, that MSW may contain high levels of Pb, Cu, Zn , Cd, Ni, Mn and Cr (Alloway, 1995), this research has the objectives to evaluate heavy metal levels in soils and plants from this agricultural region and the risks of contamination with continuous use of MSW in soil-plant system.

2. Materials and Methods

The sampling area was obtained in a guava (*Psidium guajava* L.) orchard located 90 Km from Rio de Janeiro city. This area is an Inceptsol (gleysoil), where the guava plants has received 40 $t.ha^{-1}$ of MSW since 1985 every year, on the soil without tillage. Soil samples were taken in August of 1996, at three depths : 0-50, 50-150 and 150 to 300mm. In the same experimental area, another guava orchard (control area), where there was no MSW amendment, has received the same soil sampling. Guava leaves were sampled on both areas, and fruits only on the area with eleven years of MSW.

Statistical design was a factorial with two areas and three soil depths. Total concentrations of Cd, Cr, Cu, Mn, Ni, Pb and Zn in soil samples and guava dry fruits and leaves were obtained with Cottenie (1982) method using aqua regia (HCl-HNO_3) and nitroperchloric acid digestion respectively. The analysis were performed in a Varian flame atomic absorption, model AA-800.

3. Results and Discussion

When compared to levels mentioned by, Alloway (1995) and Natale (1993), total metal levels in guava fruits and leaves in soil amended with MSW (Table 1), were below or in the range of critical levels. It is difficult to conclude about metal levels, considering that there is no specific reference levels to this species of cultivated plant and its edible part. Otherwise, when are observed the leaves, metal levels where there was no MSW amendment, was evident that guava plant had a higher absorption of metals when amended with MSW during eleven years.

The difference between total soil metal levels of both areas (Table 2) is very clear in the surface depth (0-50mm), also occurring in the intermediate depth (50-150mm). The way of MSW is amended, over the soil without incorporation (tillage) could explain this results. In the deeper depth (150-300mm), the difference between the two sampled areas to Cd, Mn and Pb levels, could suggest a higher mobility of this metal chemical forms through soil profile. Organic matter in surface depth (improved with organic amendments) could explain higher superficial metal levels through organic stable chemical forms. Meanwhile, this course-textured soil, could

improve the mobility of metal species towards soil depth, which can be a risk for groundwater.

4. Conclusions

The agricultural use of MSW in a soil management system, did not represent risk of higher accumulation of heavy metals in guava leaves and fruits above known background levels. Though, there was an increase in soil heavy metals levels with eleven years of MSW amendment, however it did not reach critical levels mentioned in scientific research. The difference observed, pointed to the necessity of a monitoring control in soils with continuous MSW use, mainly if this areas have short term culture (vegetables).

Table 1 - Total heavy metals levels in guava leaves and fruits (from three replicates) and some cultivated plants background levels.

Heavy metals in mg kg^{-1}						
Cd	**Cr**	**Cu**	**Ni**	**Mn**	**Pb**	**Zn**
Guava leaves with 11 years of MSW						
1.01	3.2	8.6	13.1	100.5	26.6	29.0
Guava leaves without MSW						
0.74	2.7	3.9	11.7	42.8	20.6	14.6
Guava fruits with 11years of MSW						
0.00	1.8	2.6	0.8	4.1	6.4	8.0
Critical metal levels in plant species (Alloway, 1995)						
4-30	2-18	5-64	8-100	300-500	30-300	100-400
Levels obtained in a brazilian guava research (Natale, 1993)						
-	-	10-16	-	130-425	-	21-23

Table 2 - Total heavy metals distribution in three soil depths in the two sampled areas (from three replicates).

Depth -cm-	Heavy metals in mg kg^{-1}													
	Cd		**Cr**		**Cu**		**Ni**		**Mn**		**Pb**		**Zn**	
	MSW (1)	Contr (2)	MSW	Contr	MSW	Contr	MSW	Contr	MSW	Contr	MSW	Contr	MSW	Contr
0-5	2.4	0.6	23	7.1	35	3.1	11	4.5	208	40	36	12	92	42
	AA	**AB**	**AA**	**AB**	**AA**	**AB**	**AA**	**AB**	*Aa*	**AB**	**AA**	**AB**	**AA**	**AB**
5-15	1.7	0.5	12	6.0	13	2.7	7.9	4.0	148	28	27	11	56	25
	BA	**AB**	**BA**	**AB**	**BA**	**AB**	**BA**	**AB**	**AA**	**AB**	**BA**	**AB**	**AA**	**ABB**
15-30	1.4	0.4	5.1	6.3	3.0	2.7	4.6	4.1	43	20	15	10	25	16
	BA	**AB**	**CA**	**AA**	**CA**	**AA**	**CA**	**AA**	**AA**	**AB**	**CA**	**AB**	**AA**	**BA**

Average followed by the same majuscule in the columns and minuscule vowel in the row do not differ to 10% of probability by test of Tukey
(1) = Soil from guava cultivated area which received MS W during 11 years
(2) = Soil from guava cultivated area which did not receive MSW anywhere

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TRACE ELEMENT VARIATIONS IN A VERTISOL OF SOUTHERN ITALY AMENDED WITH MUNICIPAL SOLID WASTE COMPOST.

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1. Introduction

Municipal solid waste (MSW) compost can be used in agriculture as amendment, after biological treatments, this could improve soil fertility and enrich soil with mineral and organic compounds (He et al., 1992; Shiralipour et al., 1992; Kraus et al., 1996). A role very important could be played by MSW also in relation to mineral nutrition of typical crops as sugar beet (SB) and durum wheat (DW). In this paper are reported the results of four years field experiment carried out in Southern Italy in a vertisol treated with repeated crop field additions of MSW compost.

2. Materials and Methods

Field trial started in 1993 in Southern Italy (Foggia) on a silty-clay soil (Typic Chromoxerert, as USDA classification) with the main characteristics: pH= 8.3; o.m.=2.07%; clay=31.1%; C/N=10; $CaCO_3$=4.3%. The field experiment was laid out on completely randomized blocks with three replications, comparing three types of fertilizer (A1: mineral fertilizer; A2: organic + mineral fertilizer; A3: municipal solid waste compost) and three N doses (B1=40 kg N ha-1; B2=80 kg N ha-1; B3=120 kg N ha-1) on sugar beet sowing in autumn (cv. Suprema) and durum wheat (cv. Simeto) in rotation, both cropped each year on two adjacent plots.

The compost obtained from the SLIA Plant in Brindisi and in Castel di Sangro through aerobic transformation of municipal solid wastes.

Soil samples were collected before and at the end of both the cropping cycles (0-40 cm depth) of all 54 elementary plots. Heavy metals were solubilized from the soil by a mixture of HCl and HNO_3 (3 : 1) and measured by atomic absorption spectrophotometry. At the harvest, yields, qualitative characteristics of different crops and mineral contents of the plant tissues were determined

In this paper, was considered the treatment with (+MSW) and the test (-MSW) without municipal solid waste compost. Cu, Pb and Zn soil content variations on cropping sequence DW-SB were recorded.

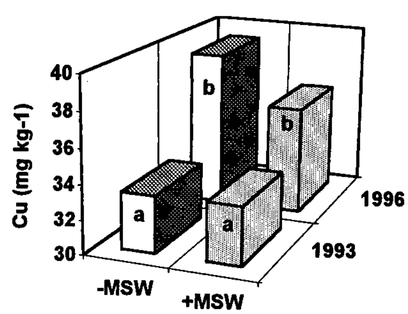

Figure 1 - Soil Cu content in the plots cropped with DW-SB sequence. (Columns with differing letters are significantly different at P<0.05)

3. Results and Discussion

In Figure 1 are reported the variations of soil Cu content in the plots cropped with DW-SB sequence. Independently from the experimental treatments the soil Cu content increases through the years probably because there was a large field spatial variability and also a seasonal variation. The supply of MSW compost did not introduce any variation to Cu total pool of the soil.

Figure 2 shows the trends recorded for soil Pb content of tested cropping sequence. The differences between experimental treatments and the years were not significant. When MSW compost was supplied, Pb content of the soil appears more constant during the trial period.

Similarly to the findings recorded for Cu soil content, also total Zn concentration increased from 1993 to 1996 independently of experimental treatments. Also for this element the large soil spatial variability (Figure 3) probably plays a more important role.

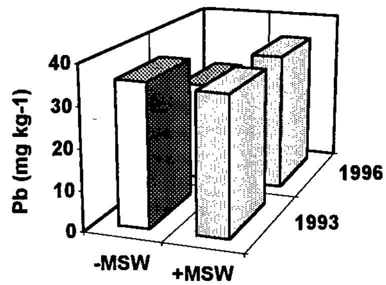

Figure 2 - Soil Pb content in the plots cropped with DW-SB sequence.

4. Conclusions

Evaluating the observed variations in total Cu, Pb and Zn contents of the soil it seems that the application of MSW-compost on a typical Vertisol of southern Italy did not pose serious risks of environmental pollution. Naturally the results obtained must be considered only tentatively and dependent on mineral composition of MSW-compost that was incorporated in the soil as agronomical doses (from 2 to 15 t ha^{-1}).

Using municipal solid wastes compost in agriculture allows their valorization and give rise to sustainable positive effects on soil fertility.

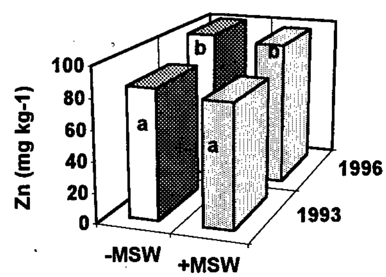

Figure 3 - Soil Zn content in the plots cropped with DW-SB sequence.

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SPECIATION AND MOVEMENT OF SELECTED HEAVY METALS IN LONG-TERM POULTRY WASTE-AMENDED SOILS

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1. Introduction

Typically, poultry waste (a combination of bedding material and excreta) is intensively applied to relatively small areas of land. Metals are added as nutrients to poultry diets leading to their occurrence in the waste stream. High concentrations of heavy metals in waste-amended soils create environmental concerns due to potential pollution of surface and groundwater via run-off and leaching as well as potential phytoxicities to plants (KINGERY et al., 1994). The objective of this study was to determine accumulation of heavy metals in solid-phase fractions within the soil profile of a 25-year waste-amended soil.

2. Materials and Methods

Surface soils (0-20 cm) were taken from a waste-amended pasture and adjacent forested soils. Both soils were clayey, mixed, thermic Typic Hapludults with shale parent material. Soil profiles were also sampled to 200-cm depth from both soils. A sequential selective dissolution procedure, generally based on the procedures developed by TESSIER et al. (1979) and HAN and BANIN (1997), which divided metals in the soils into six operationally defined solid-phase fractions, was used for determining heavy metal concentrations.

3. Results and Discussion

Copper, Zn and Mn accumulated in the poultry waste-amended soil over 25 years (Figure 1a). Copper and Zn concentrations in the amended soils were 21 and 7.5 times higher than those in the forested soil, respectively. The total concentrations of Cu and Zn in the amended soils increased at an approximate rate of 2 mg $kg^{-1}yr^{-1}$. Nickel and Cr concentrations in the amended soils were also slightly greater than forested soil. Lead concentration was similar in both soils.

Copper in the amended soil was mostly present in the organic matter fraction (50%), while Zn was found in the easily-reducible oxide fraction (50%).This suggests that Cu and Zn in this long-term amended soil is potentially bio-available.

Most of the metals applied to soils with poultry waste accumulated in the upper layer (0-10 cm) (Figure 1b). The total concentrations of Cu and Zn in the surface forested soils were lower than subsoil. In the lower part of the profile of poultry waste-amended soil (100-180 cm), total metal concentrations, especially for Zn, were much higher (nearly 2 times) than those in the forested soil. Furthermore, Zn and Cu bound in the iron oxide (especially, crystalline iron oxide) and clay mineral fractions were considerably higher within the profile in the poultry waste-amended soils compared to the forested soil (Figure 1c). This indicates that metals in the poultry waste-amended soils, after long-term annual applications, were subject to transport within soil profiles and were associated with iron oxides and fine clay particles. These data suggest the possibility that metals may be transported via association with mobile colloids.

4. Conclusions

After long-term of applications of poultry waste, Cu and Zn accumulated in the surface soils and they were potential available to plants. Further, Zn transport even in this clayey soil profile was observed. High bioavailability, along with considerable accumulation and high mobility of heavy metals (Zn) in poultry waste-amended soils suggests that regulation of poultry-waste application should not only consider P and N inputs, but also heavy metals.

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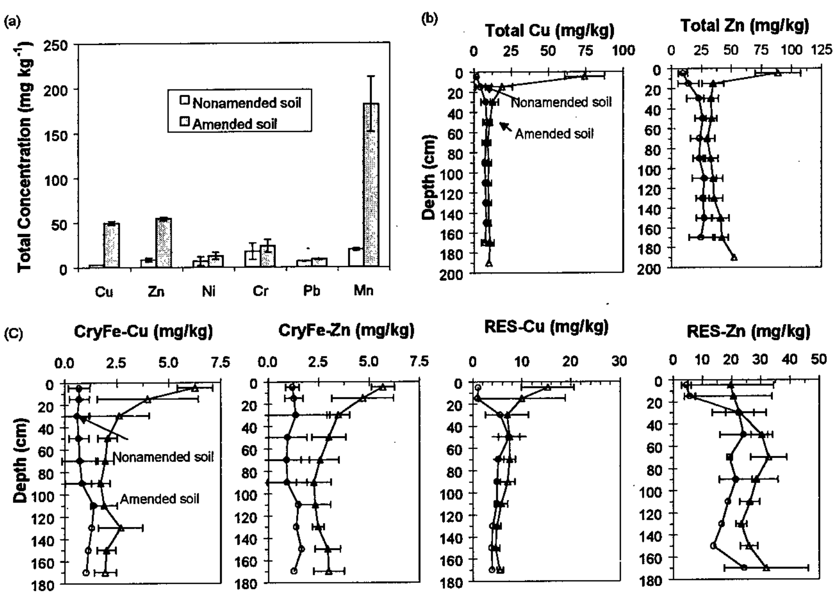

Figure 1: (a) Total concentrations of heavy metals (extracted with 4M HNO_3) in the poultry waste amended soil and adjacent forested soil (n=10). (b) Soil profile distribution of total concentrations of Cu and Zn (n=8 and 6 for amended and nonamended soils, respectively). (c) Soil profile distribution of Cu and Zn bound in the crystalline Fe oxide (CryFe) and in the clay mineral fractions (RES) (n=5 and 2 for amended and nonamended soils, respectively).

REMEDIATION OF MIMICKED CADMIUM CONTAMINATED SOIL USING COAL FLY ASH STABILIZED BIOSOLIDS

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1. Introduction

The availability of heavy metals is pH-dependent and at an alkaline pH of >7, heavy metals are mainly in insoluble and immobilized forms. Phytostabilization can be achieved through amending soil with alkaline materials such as lime and coal fly ash (Iskandar and Adriano, 1997). An alkaline biosolids constituted of coal fly ash and lime stabilized biosolids was demonstrated to be a good soil conditioner for acidic soils (Wong, 1998). Hence, it is the aim of the present study to use this alkaline biosolids as a soil ameliorant to reduce the availability of heavy metals in order to facilitate the establishment of *Agropyron elongatum* in Cd contaminated soils.

2. Materials and Methods

The mimicked Cd contaminated soil was prepared by mixing an acidic sandy loam with 0, 5, 20 and 50 mg Cd kg^{-1} soil in the form of nitrate salt. The pH of mimicked Cd contaminated soil was adjusted to 4.5, 5.5 and 7.5 by adding various amounts of coal fly ash-lime-sludge mixture (CLS). Controls including soil without any amendment and soil being limed to pH 4.5 were also prepared for comparison. Soil samples were collected from each treatment for determination of pH, EC and DTPA-extractable Cd. Then 100 seeds of *A. elongatum* were sown in each pot and plants were allowed to grow for a period of 12 weeks. All control treatments received a complete fertilizer in order to assess the nutrient availability of the CLS. After harvesting, dry weight yield of shoot and root, and tissue Cd contents were measured.

3. Results and Discussion

pH and EC of soil increased with an increase in the amount of CLS amendment. The alkaline biosolids amendment significantly reduced the DTPA-extractable Cd contents, which decreased according to the CLS application rate owing to the increase in pH (Figure 1). Alkaline biosolids

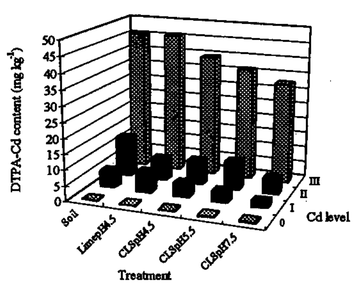
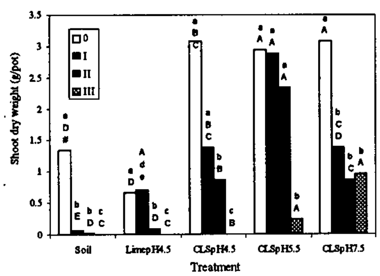


Figure 1. DTPA-extractable Cd concentration of Cd-spiked soil with pH adjusted to 4.5, 5.5 and 7.5 by lime and CLS amendment.

amendment was more effective in reducing Cd availability than lime amendment especially at the highest concentration of Cd contamination which is likely due to the increase in soil organic matter.

Seed germination of *Agropyron* was significantly inhibited with an increase in Cd level in soil and also at the highest level of CLS amendment owing to the increase in soil salt content. Shoot and root dry weight yields decreased with an increase in Cd concentration at various soil pH but the CLS amendment significantly improved the yield at each level of soil Cd contamination as compared to the control and the limed soil at pH 4.5 (Figure 2).



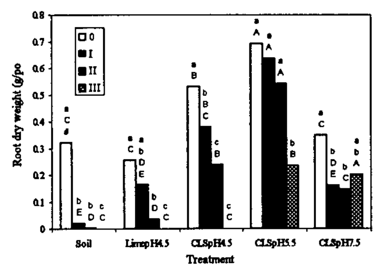


Figure 2. The shoot and root Cd concentrations in *A. elongatum* grown in CLS amended soil spiked with various concentrations of Cd. Columns with same small letter do not differ significantly among the various concentrations of Cd within the same treatment group while column with the same capital letters do not differ among the various treatment groups receiving the same conc. Of Cd at $p<0.05$ according to the Least Significant Difference test.

This is likely due to the dilution effect and the increase in organic matter following CLS amendment, which might reduce the availability of Cd in soil. The CLS had an additional benefit concentrations. in increasing soil nutrient contents derived from sewage sludge. A substantial yield could be maintained at a soil Cd concentration of 20 mg kg^{-1} with pH adjusted to 5.5 by CLS amendment. Tissue Cd concentration increased with an increase in soil Cd contents but decreased with an increase in CLS amendment rates (Figure 3). However, the highest removal percentage of Cd was only 3.12% indicating that *Agropyron* did not have the ability to extract Cd from the Cd contaminated soil.

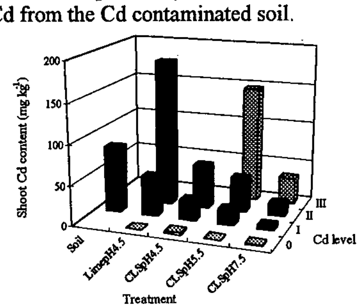


Figure 3. Shoot Cd concentrations in *A. elongatum* grown in CLS and lime amended soil spiked with various Cd concentrations.

4. Conclusions

It can be concluded that CLS amendment was effective in reducing the metal availability through alkaline stabilization and providing better plant growth condition. It is recommended that CLS can be used to adjust the acidic soil pH to 5.5 – 6 to reduce the availability of Cd and to facilitate the growth of *Agropyron*.

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IMPACTS OF HEAVY METAL CONTAMINATED SEWAGE-SLUDGE ON *RHIZOBIUM LEGUMINOSARUM* BIOVAR. *TRIFOLII*

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1. Introduction

Sewage sludge is a resource, containing high concentrations of plant nutrients, and its recycling to land is an accepted agricultural practice throughout Europe and North America. However, due to its contaminant content, its disposal is subject to numerous rules and guidelines governing its heavy metal concentrations. European research has indicated that the symbiotic relationship between white clover (*Trifolium repens* L.) and *Rhizobium leguminosarum* biovar. *trifolii* is sensitive to heavy metals in soil. Our aim is to develop a simple tool to provide a "yes/no" assessment of the nitrogen fixing status of a clover sward growing on sewage sludge amended soil and, thereby, to provide an effects-related index of adverse impact for use in the development of environmental standards and guidelines.

2. Materials and Methods

Three approaches are currently being assessed for determining the impact of heavy metal-contaminated sewage sludge on the nitrogen fixing ability of clover. These are:

a) Use of a $\delta^{15}N$ natural abundance (NA) technique to assess the N-fixing capacity of field nodulated clover.
b) A clover infection test to determine the presence or absence of effective strains of *R.leguminosarum* bv. *trifolii* in soil samples using a technique first described by Vincent (1970).
c) Use of a bacterial bioassay which measures the inhibition of light production from *lux*-gene modified *Rhizobium*.

3. Results and Discussion

The relevance of each of the 3 approaches taken in this project, to the capacity of sewage sludge treated soils to maintain a viable rhizobial population has been assessed. The NA method has the potential to provide reliable and relatively easily performed assessments of N_2 fixation by field-grown legumes. The clover infection technique has been successfully used to assess the effects of soil contaminants on populations of *R.leguminosarum* bv. *trifolii* in soil both in this project and in other studies (Giller *et al.*, 1989 and Smith, 1997). This test also shows, in a rudimentary way, whether nitrogen is fixed in the nodules. Ineffective nodules are white and the host plant growth is poor whereas effective nodules are pink due to the presence of leghaemoglobin (a pigment produced only in nitrogen-fixing nodules) and plant growth is vigorous. *Lux*-gene modified *Rhizobium* bioassays have been carried out using pure salt solutions of Zn, Ni and Cu (three of the elements most likely to limit the use of sludge in agriculture, given the current legislation). Results show that *Rhizobium* is an excellent biosensor for acute soil ecotoxicity testing at environmentally relevant metal concentrations (Figures 1-3).

Figure 1: Response of *Rhizobium* to Zn

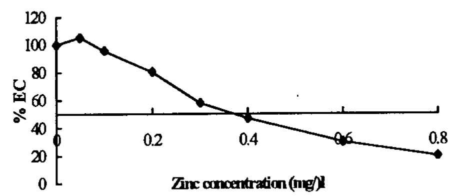


Figure 2: Response of *Rhizobium* to Ni

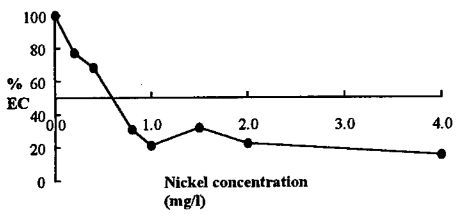
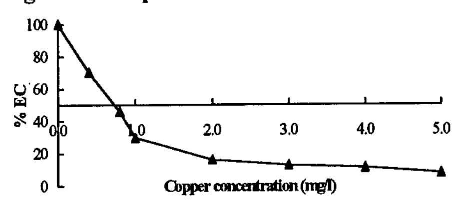


Figure 3: Response of *Rhizobium* to Cu



4. Conclusions

It has been recommended by European research (Giller *at al.,* 1998 and Giller e*t al.,* 1989) that a simple screening for the presence of rhizobia which could nodule white clover would be a rapid method for the screening of soils for toxic effects of heavy metals. Application of the three approaches taken here may provide such a method, in addition, the *lux*-bioassay allows the determination of the bioavailablity of metals in soil to a target population (e.g. rhizobia).

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IMPACT OF SEWAGE SLUDGE ON HEAVY METAL CONCENTRATIONS OF AGRICULTURAL SOILS IN LUXEMBOURG

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1. Introduction

Due to its beneficial action on soil structure and nutrient status, sewage sludge is commonly recycled as amendment for agricultural soils. However, abundant loads of heavy metals can present a danger to the ecosystems by leaching and thread to consumers by plant uptake. In order to investigate the impact of sewage sludge on the pedosphere, the heavy metal contents of various agrarian soils fertilised with sewage sludge were compared to soils amended with traditional manure and mineral fertilisers.

2. Materials and Methods

Four categories of agricultural soils were investigated: sandy soils situated on sandstone; clayey soils with a low pH situated on lias; clayey soils with a high pH situated on lias; stony soils situated on Devonian shale. Soil samples of the ploughed layer were collected in May 1997 at different sites under crop (maize) production. After harvesting, additional soil samples were collected at different depths. For total heavy metal content determination, all samples were digested in aqua regia. The bioavailable fraction was estimated after an extraction with DTPA. All samples were analysed for Cr, Cu, Ni, Pb and Zn on a AA spectrometer. Fe, Al and Mn contents were determined by colorimetric analysis. Soil pH was determined in $CaCl_2$ extracts. For each soil category, plots having always been fertilised with traditional manure and mineral fertiliser, as well as plots having been amended for several years with municipal sewage sludge were sampled.

3. Results and Discussion

Concerning the total heavy metal concentrations, no significant difference was observed between the two types of amendments. Nevertheless, the total content of metals varied according to the studied soil type, with the highest trace element concentrations for the stony soils situated on shale (example of copper in Figure 1). A principal component analysis showed that the total concentrations in trace elements are best explained by iron and organic matter contents and less well by pH. Presuming that the iron concentration varied according to the substrate, this analysis underlined the importance of the substrate for the assessment of the total metal composition. The bioavailable fraction was highest in the sandy soils for copper, zinc and nickel (percentage of the total content). This trend could not be observed for lead. These results confirmed the hypothesis that, on the contrary to lead, the mobility of copper, zinc and nickel was increased by a low pH. Again, no significant difference existed between the two types of amendments. In order to determine whether the different plots were subjected to pollution or not, the trace element levels at different depths were compared using the strategy described by BAIZE (1997). This method

revealed the presence of contamination both for sites fertilised with traditional amendments and sewage sludge sites.

4. Conclusions

Our results do not allow to distinguish between the sewage sludge and traditional amendments. According to WILCKE and DOHLER (1995), agricultural activity always implies an anthropogenic pollution of the soils and, in our case, this contamination is weakly correlated to the type of fertiliser used. On the other hand, we could show that the total concentrations of heavy metals are strongly related to the substrate and determine the normal trace element composition for each soil type. In the Grand Duchy of Luxembourg, the sandy soils present the lowest trace element levels, whereas the stony soils on shale show the highest concentrations.

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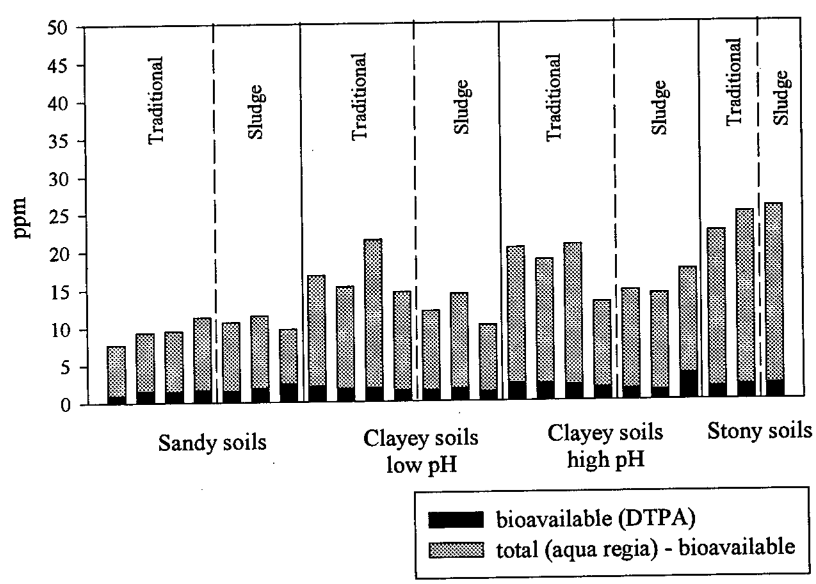
Figure 1: Copper contents of various agrarian soils



TRANSFER OF ZINC FROM DREDGED TOXIC SEDIMENTS TO A SOIL : FIRST CHARACTERIZATION OF THE SEDIMENT

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1. Introduction

In low regions, sediments are usually accumulated in rivers; to preserve boat circulation, it is necessary to periodically dredge these materials which can be disposed on soils along banks; it's the case in France for instance. In industrial or agricultural areas, sediments can be polluted by huge heavy metal contents and such deposits can constitute a risk for the environment. In order to assess the potential transfer of pollutants to the subsoil and the water table, a field approach was conducted on a site of North of France: 50 m^3 of sediments strongly contaminated by heavy metals, were dredged from a canal and deposited on a clean agricultural soil. The site is equipped to follow the composition of the infiltrating water and the groundwater. Metal distribution in the sediment deposit and along the underlying soil profile was determined every three months and followed three years. In this work, we especially report the initial characterization of sediments and the mineral determination including the distribution of Zn, Pb and Cd in distinct grain size and density fractions.

2. Materials and Methods

Samples were dried at 25°C and sieved at 2 mm. Total element contents were determined by ICP after hot acid digestion and XRD analyses specified the mineral composition.
In other respects, a physical approach was carried out to separate the particle fractions using wet sieving (500, 200 and 50 µm), density fractionation (d=2,9) and a separation based on the forms and colours of particles. The different fractions were analysed by ICP and characterized by XRD and Environmental Scanning Electronic Microscopy (ESEM-EDS) which permit to observe and analyse the surface of the particles in their natural state. It is thus possible to define some heavy metal enriched fractions.

3. Results and Discussion

Characterization and total element contents are gathered in Table 1.

	Sediment	Soil
pH	7,2	8,3
Organic C (%)	10	1
Minerals (XRD)		
Quartz	+++	+++
Calcite	++	++
Albite	+	++
Microcline	+	+
Iron oxides	+	+
Clay minerals	++	+++
Kaolinite	++	++
Illite	++	+++
Smectite	+	++
Chlorite	+	+

Element (mg/kg)	Sediment	Soil
Zn	6000	46
Cd	160	<1
Pb	600	18
Ca	50000	37500
Na	2000	6700
K	7800	14800
Al	28000	40600
Fe	17000	21000
Mn	230	520
Mg	2300	3900

Table 1 : General characterization and total element contents in soil and sediment

These results show that sediments are strongly contaminated in Zn, Pb and Cd whereas soil contents are similar to the local background. Thus, in case of metal migration from the sediments to the underlying soil, the transfer should be pointed out. We observe (Figure that the finest fraction (<50 µm) concentrates the most part of metal stock (75% of Zn and Pb, 85% of Cd) even if the coarsest one (500-2000µm) presents very high concentrations of metals as well (8000 ppm of Zn, 75 ppm of Cd and 850 ppm of Pb). So, it is interesting to focus on these two highly polluted fractions in order to study a possible transfer and a remobilization of metals.

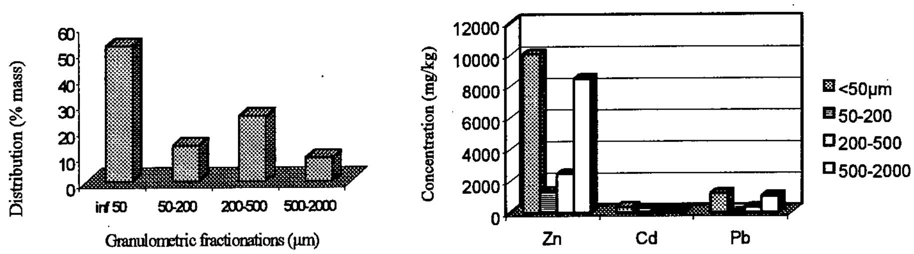

Figure 1 : Grain size distribution and metal contents in the sediment.

The observation of the 500-2000 µm fraction with binocular lens shows a major part of anthropogenic materials, including spherical slags. Their ESEM-EDS analyse indicates that Zn and Cd are predominantly associated with S at the surface of a silicate matrix. Pb couldn't be pointed. XRD analyses of the sediment heavy fraction obviously show metallifereous minerals: ZnO, ZnS and $PbCO_3$. The occurrence of PbS and Zn_2SiO_4 is probable. ESEM-EDS analyses as well indicate several associations with Zn : Zn and O with or without Fe, Zn with S and finally Zn with Si. Small particles (10µm) of Pb and PbS are also identified with the silicate matrix.

4. Conclusions

These first results allowed us to identify two rich metal grain size fractions in the sediment: the finest and the coarsest. The 500-2000µm fraction contains slag particles: ESEM-EDS analyses show associations of Zn-S and Cd-S. A density separation of the sediment permitted to isolate metallic minerals (ZnS, ZnO, $PbCO_3$ and, probably, $ZnSiO_4$ and PbS). Following XRD and ESEM-EDS analyses, an EXAFS study will be undertaken at the ESRF of Grenoble in order to specify the evolution of Zn speciation after the deposit.

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THE DISTRIBUTION OF CD IN FIVE FRACTIONS OF SEWAGE SLUDGE AFTER EIGHT MONTHS AEROBIC AND ANAEROBIC INCUBATION

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1. Introduction

The total content of heavy metals can give little information about the processes of the availability of elements in sewage sludge. Metals in sludge are found in five major forms: (i) as simple or complexed ions in soil solution; (ii) as easily exchangeable ions; (iii) organically bound; (iv) coprecipitated with metal oxides, and other secondary minerals; or (v) as ions in crystal lattices of primary minerals. Metal ions present in the first three forms are considered to be the highest available to plants, successive forms representing a decreasing degree of availability (Lake et al.,1984). The effect of aerobic and anaerobic incubation of fresh sewage sludge treated by lime, bentonite and organic material on the changes of Cd bound in five fractions was investigated.

2. Materials and Methods

Three sewage sludges differing in general properties and containing 0.95-6.09 mg.kg^{-1} of Cd in dry matter were mixed thoroughly in triplication with a) lime 18% w/w of dry sludge, b) bentonite 30% w/w of dry sludge, c) peat and straw ratio 50:5:45% w/w/w of dry sludge. All treated sludges were incubated under aerobic (air conditioned each 14th day) and anaerobic conditions at 21 °C. Non treated sewage sludge was incubated at the same conditions. The samples taken after eight months were extracted to follow BCR EUR 14763 EN modified extraction scheme (Ure et al.,1993) and analysed for Cd by flame and graphite furnace AAS. Analyses of total element content followed Tlustos et al.(1994) procedure. Certified reference material RM 12-03-12 Sludge was used for quality control.

3. Results and Discussion

The Cd distribution was significantly affected by the type of incubation. Due to the oxidation of organic matter the total Cd concentration was higher in incubated sludges compared to fresh one (Fig. 1). Majority of Cd (> 98 %) was bound in the most stable organic and mineral compounds in the fresh sludge. The incubation changed the distribution of Cd. Sludge under anaerobic conditions contained 85 % of Cd in the low soluble fractions. In aerobic conditions Cd was released and only 43 % was bound into organic and mineral fractions. Highest increase of Cd was in Fe+Mn oxides fraction under both incubation and in available fraction under aerobic conditions.

The addition of lime, bentonite and the mixture of straw with peat changed the Cd distribution into individual fractions (Fig. 2). Lime application stabilized sludge and hold the Cd in the

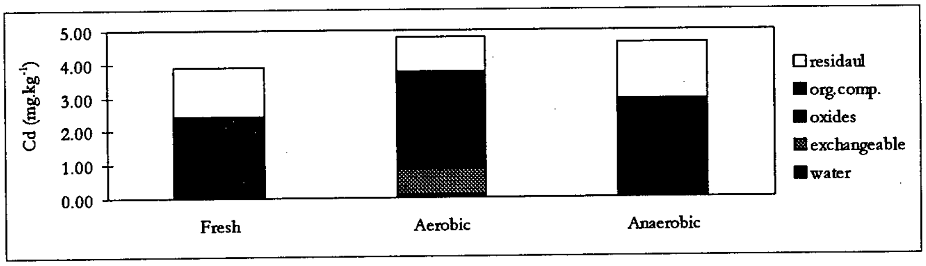

Fig. 1: The content of Cd (mg.kg^{-1}) in main fractions of treated and incubated sewage sludge.

lowest soluble fractions in both differently incubated sludges. The other two materials showed the different pattern under aerobic and anaerobic conditions. Aerated samples led to movement of Cd into the easily available fractions at both treatments. Application of straw and peat partly subdued anaerobic environment and the treatment showed the same pattern as at aerobic one, but the amount of released Cd was lower. Bentonite did not change Cd distribution in anaerobic conditions.

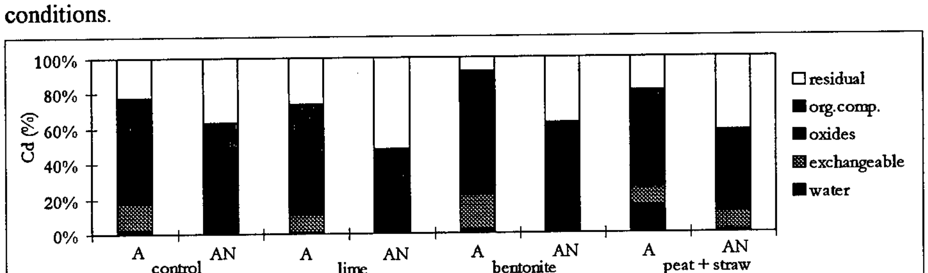

Fig. 2: Relative changes of Cd content (%) in main fractions of incubated sewage sludge treated by different materials (A= aerobic; AN= anaerobic).

4.Conclusions

Fresh sludge contained Cd mostly in organic and residual fractions. Anaerobic incubation changed the distribution slightly, but aerobic environment led to Cd movement into easily released fractions. Among tested material only lime decreased Cd avalability in both types of incubation.

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BIOSOLIDS AS ZINC SOURCE FOR SOYBEAN

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1. Introduction

The Argentinean Pampas are characterized by its very high soil fertility and for more than one hundred years agriculture was based on the soil nutrient supply capacity. As a result, agricultural soils were nutrient depleted and are now deficient or poorly supplied in several nutrients, among them zinc, boron and to a lesser extent copper (Lavado and Porcelli, 1998). Fertilization is a recent issue and sewage sludge is starting to be used in the area.

It is widely known that the long term application of biosolids tends to increase the zinc and other trace metals content in soils and in crops. Our objective was to analyze the short term biosolid application as a micronutrient source.

2. Materials and Methods

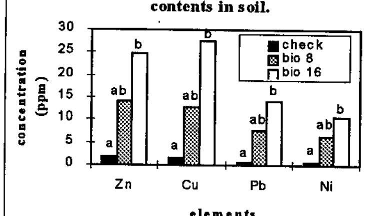
Biosolids from treatment plants of Buenos Aires city and from the outskirts were used. The field experiment was carried out in 1996/97 near the Chacabuco town. The soil was a very degraded Typic Argiudoll, which had lost most of the A horizon. Three treatments were compared: Check; Biosolid 1: 9 $Mg.ha^{-1}$ DM and Biosolid 2: 18 $Mg.ha^{-1}$ DM. The experiment was designed in randomly blocks with three replications,

Soil Bioavailable Cd, Cr, Cu, Mn, Ni, Pb and Zn were extracted with DTPA and B was extracted with hot water. The same elements in plants were extracted by acid extraction (nitriv and perchloric acid). All determinations were performed by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). Other determinations were performed using standard methods.

3. Results and Discussion

The average trace metal concentration of the used biosolid was lower than standard regulations (i.e. USEPA, 1995). The concentration of Zn was 1300 ppm. The biosolid application caused an increase in the soil pH (5.8 to 6.6). Tsadilas et al. (1995), has indicated decreases as well as increases in soil pH, using also non limed biosolid applications. Exchangeable calcium increased but potassium and magnesium do not change. As expected, the biosolid treatments showed higher contents of nitrogen, phosphorus and sulfur.

Fig 1: Zn, Cu, Pb and Ni contents in soil.



The application of the higher biosolid doses caused significant increases in bioavailable Cu, Ni, Pb and Zn, as related with the check (Fig 1). The content of Zn in the soil prior the application of biosolids was around the limit of deficiency (1.8 ppm). The bioavailable level of Zn increased and Cu, Ni and Pb, followed a similar pattern. Manganese, B and Cd bioavailable concentrations did not show significant differences among treatments.

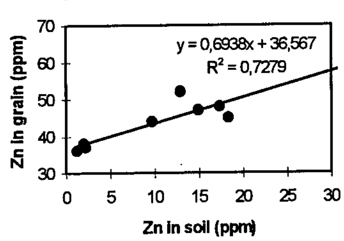
Soybean yield increased as a response of biosolid application. In grains only Zn

concentration increases; there were, however, significant differences between the check and biosolid doses but not between the later.

	Zn	*Cu*	*Pb*		*Mn*	*Ni*	*B*	*Cd*
				Grain				
Check	37.0	10.7	<5		23.3	3.3	55.0	<0.5
Bio 1	46.3	11.7	<5		27.3	3.7	50.0	<0.5
Bio2	49.3	12.7	<5		27.3	5.3	64.0	<0.5
				Leaves				
Check	27.0	6.7	<5		93.0	2.0	55.3	<0.5
Bio 1	50.0	7.7	<5		111.3	3.0	50.0	<0.5
Bio2	50.0	7.7	<5		85.0	2.0	64.0	<0.5

Despite the increases in soil concentrations there was not any accumulation of Cu, Ni and Pb either in soybean leaves or in grains. In the rest of the trace elements studied, the plant concentration did not differ among treaments. A linear correlation between the Zn concentrations in leaves and grains (fig.2) and the bioavailable Zn soil concentration was found. The high correlation suggests that the crop absorbed most Zn from the biosolid. Both plant phosphorus and zinc concentrations increased, following the treatments, but the ratio P/Zn changed, modifying the antagonistic effects of P on Zn absorption and then in the yields (Goto et al., 1993).

Fig 2: Zn contents in soil and grain



4. Conclusions

Considering the general Zn deficiency of soils, and its relationship with other nutrients, biosolids could be considered good fertilizers in short term applications.

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CHROMIUM IN SOIL AND SORGHUM PLANTS AS AFFECTED BY SEWAGE SLUDGE ENRICHED WITH THE METAL

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1. Introduction

Sewage sludge is a residue from the wastewater treatment that presents in its composition organic matter and nutrients for plant growing, so that its use in agriculture would resolve two great problems: a way of sewage sludge disposal and a decreasing in the use of mineral fertilizers, another mechanism of environment pollution.

But, sewage sludge contents heavy metals in its composition, among them the chromium, which also are causes of pollution. Thus, the objective of this work was to estimate the effect of sewage sludge, when enriched with increasing rates of chromium, on the availability of the chromium to sorghum plants growing under field conditions.

2. Materials and Methods

The trial was carried out in Jaboticabal county, State of São Paulo, Brazil, in a Typic Haplorthox type soil (OM= 26 g kg^{-1}, pH ($CaCl_2$ 0.01 mol L^{-1})= 4.7, K= 3.2, Ca= 14, Mg= 4, H+Al= 38 $mmol_c$ dm^{-3}, sand= 640, silt= 60, clay= 300 g kg^{-1}) under field conditions. Sewage sludge (K= 108.33, Ca= 150.00, Mg= 216.67, Cu= 9.33, Fe= 3,941.67, Mn= 17.42, Zn= 8.42, Cr= 8.58, Cd= 0.33, Ni= 6.25, Pb= 9.58 mg kg^{-1}), supplied by SABESP (Suzano, SP) was air-dried, ground to 40 mesh and enriched with chromium ($CrCl_3.6H_2O$) in the rates 0 (without addition of Cr), 100, 300, 900 and 2700 mg kg^{-1} sewage sludge. A liming was made 148 days before sowing to elevate the V% value to 70% (3 Mg ha^{-1} dolomitic limestone). Sewage sludge, so prepared, was applied to the soil, in the furrow, in the rate of 40 Mg ha^{-1}. Sorghum was then cropped in a density of 140,000 plants ha^{-1}. The experimental design was a randomized blocks with four replications. Soil was sampled 64 and 108 days after sorghum sowing at the depths 0-10 and 10-20 cm. The samples were air-dried, sieved to 2 mm and analyzed for chromium in the nitric-perchloric and in the DTPA extracts. Plants were sampled at 64 (only the leaf indicated for nutritional diagnosis) and 108 days after sowing (leafs, stalks and panicles). Plant material was washed with 0.1 mol L^{-1} HCl, water, distilled water and deionized water, dried at 60-70°C, ground to 40 mesh and analyzed for chromium in the nitric-perchloric extract.

3. Results and Discussion

In the sampling at 64 days after sowing, the content of chromium in the leafs was not affected by the rates of chromium in the sewage sludge. The rates of chromium also did not affect dry matter production and the metal concentration in the different parts of the sorghum plants, when the sampling was made 108 days after sowing (Table 1).

The content of chromium in the nitric-perchloric and in the DTPA extracts in the soil samples obtained 64 days after sorghum sowing increased with the rate of chromium added to sewage sludge (Table 2).

Table 1. Dry matter production and chromium content in different parts of sorghum plants at 108 days after sowing.

Cr Added (mg kg^{-1})	Leaf	Stalk	Panicle
	Dry Matter (g plant^{-1})		
0	6.75	16.34	3.87
100	8.72	15.64	4.08
300	6.93	16.38	3.86
900	6.13	13.98	2.97
2700	7.13	15.30	3.55
	Chromium (mg kg^{-1})		
0	1.53	2.59	1.50
100	2.27	2.50	1.39
300	2.04	2.41	1.38
900	2.46	2.73	1.24
2700	1.82	2.78	1.35

In this sampling, chromium content in the nitric-perchloric extract concentrated in the layer 0-10 cm and the content in the layer 10-20 cm was not affected by the rates of the metal. At this time, chromium concentration in the DTPA extract increased with the rates of the metal and it was higher in the layer 10-20 cm. The content of chromium in the nitric-perchloric extract in the second sampling (108 days after sowing) was lower than in the first one and it was not affected by the rates of the metal added to sewage sludge or by the sampling depth.

Table 2. Chromium in the nitric-perchloric and DTPA soil extracts 64 days after sorghum sowing.

Cr Added (mg kg^{-1})	Sampling Depth	
	0-10 cm	10-20 cm
	Nitric-Perchloric Extract (mg kg^{-1})	
0	67.60 Ca	55.92 Aa
100	89.78 Bca	65.33 Aa
300	110.65 Aba	69.33 Ab
900	114.75 Aba	63.33 Ab
2700	135.95 Aa	68.53 Ab
	DTPA Extract (mg kg^{-1})	
0	6.67 Ba	3.79 Ca
100	15.10 Aba	7.37 Bca
300	15.83 Aba	16.70 Ba
900	25.75 Aa	18.28 Ba
2700	23.22 Ab	45.63 Aa

4. Conclusions

In the sampling at 108 days after sowing, the content of chromium in the plants, in the nitric-perchloric extract and in the DTPA extract was not affected by the rates of chromium in the sewage sludge.

5. Acknowledgement

The authors tanks to FAPESP, for the financial support, and to SABESP, for supplying the sewage sludge.

HEAVY METALS ACCUMULATION IN SUGARCANE PLANTS AS AFFECTED BY SEWAGE SLUDGE.

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1. Introduction

Nutrient recycling from sewage sludge by agricultural farms is of great interest because it contributes to reduce the environmental pollution. However, depending on the residual waters it is originated, the sewage sludge can present in its composition heavy metals in levels that can represent risks to contamination of soils, underground waters, plants and animals, making possible the insertion of those elements in the alimentary chain. The present work had the objective of evaluating, under greenhouse conditions, the effects of the increasing rates of sewage sludge, associated with two levels of mineral fertilizers on the heavy metals of sugarcane plants.

2. Materials and Methods

The experiment was carried out under greenhouse conditions to evaluate the effects of sewage sludge increasing rates on the heavy metals accumulation in sugarcane plants. The sugarcane variety was SP 70-1143. The soil was a *Thypic Haplorthox* (P-resin = 40 mg dm^{-3}, O.M. = 24 g kg^{-1}, pH_{CaCl2} = 3.8 and K = 1, Ca = 3, Mg = 1, H+Al = 64, CEC = 69, all in $mmol_c$ dm^{-3}, Cd = n.d., Cr = 1, Cu = 2, Fe = 310, Mn = 3.3, Ni = n.d., Pb = 1.45, Sn = n.d., Zn= 3.05, all in mg dm^{-3}). The soil was limed with roasted dolomite calcareous to increase the basis saturation to 60%. The treatments were: 0, 40, 80 and 160 t ha^{-1}, dry basis, of sewage sludge (supplied by SABESP, Suzano County, State of São Paulo, Brazil), associated with 50 and 100% of mineral fertilizer recommended to sugarcane. The elemental composition of sewage sludge was $C_{oxidable}$= 53.2, N = 5.5, P = 3.5, K = 3.8, Ca = 42.3, Mg = 7.1, S = 7.0, all in g kg^{-1}, Cd = 8.0, Cr = 579, Cu = 625, Fe = 44,450.0, Mn = 14.0, Ni = 346.0, Pb = 217.0, Zn= 1,125.0, all in mg kg^{-1}, Moisture = 740 g kg^{-1}. Fifteen days after plant emergency from small rhizomes in organic earth, the seedlings were transplanted to pots with 20-L capacity, containing soil and sewage sludge. After 120 days, plants (shoot and root) were collected, dried (60-70°C at constant weight), weighted, milled (Willey mill) and analyzed in relation to Cr Cu, Fe, Mn, Ni, Pb and Zn, after nitric-perchloric acid digestion, by atomic absorption spectrophotometer. The experimental design was randomized blocks with 3 replications. The results were organized in a factorial scheme 4 x 2 (4 sewage sludge rates and 2 levels of mineral fertilizers) to statistical analysis.

3. Results and Discussion

Sewage sludge increased the heavy metals content in shoot and roots. The shoot of plants that received 160 t $ha^{-1}$of sewage sludge accumulated 137% more Cr, 256% more Cu, 79% more Fe, 163% more Mn, 88% more Pb and 363% more Zn than shoot of plants cropped in soil that did not receive this residue. Except for Mn, the absorption of heavy metals by root was larger than by shoot. Mn detected in the shoot was larger than the one of the root, indicating that this element was transported to shoot in sugarcane plants. On the other hand, Ni was just detected in the root. Its is an indication that some plant protection process would exist to retain the element in the shoot. It is verified that the increasing rates of sewage sludge from 40 to 80 Mg ha^{-1}did not provide significant elevation in the metals accumulation.

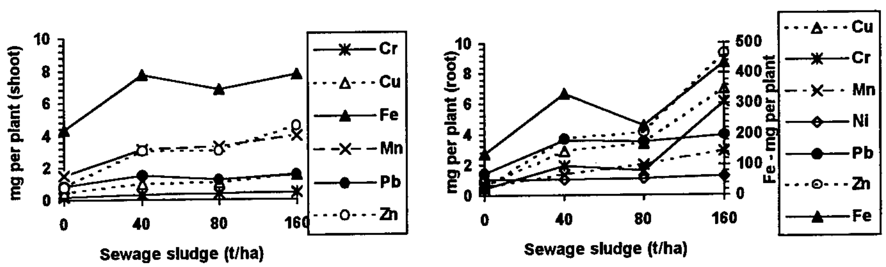

Figure 1: Heavy metals accumulation in shoot and root of sugarcane plants cropped in soil amended with increasing rates of sewage sludge.

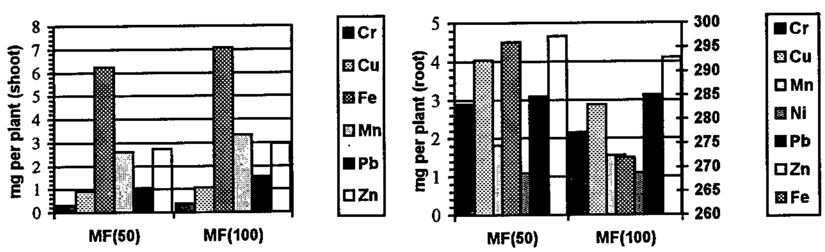

Figure 2. Heavy metals accumulation in sugarcane plants (shoot and root) affected by two levels of mineral fertilizer (50 e 100%) in the presence of increasing rates of sewage sludge.

For roots, in general, sewage sludge rates increased the heavy metals accumulation. The increases varied from 26% for Ni to 1800% for Zn. In roots, the reduction of the mineral fertilization in 50% increased the Cr, Cu, Mn and Zn accumulation in sugarcane plants. Fe, Ni and Pb contents it stayed without alterations. In the shoots there was reduction of Cr, Mn and Pb contents. Cu, Fe and Zn contents it stayed without alterations.

SEWAGE SLUDGE AS COMPONENT OF RATION TO BOVINES

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1. Introduction

The treatment of wastewater results in the production of a residue called sewage sludge, which presents in its composition organic matter and minerals that are essential for animal nutrition, so that its use as a component of animal ration is something to be studied.

The objective of this work was to estimate the effect of sewage sludge, as component of the ration for bovines in the early stages of growing, considering ration intake, gain of weight and the accumulation of heavy metals in blood and muscle.

2. Materials and Methods

The experiment design was a totally randomized with two treatments (rations with no sewage sludge and with 20% of sewage sludge) and seven replications. Both rations were equilibrated in relation to energy and protein The ration without sewage sludge contained: cotton chaff= 11.0%, soybeen chaff= 5.0%, milled corn= 32.5%, wheat chaff= 18.0%, powder molasses= 2.0%, mineral 2.0% and integral corn= 29.1%. The ration with sewage sludge contained: cotton chaff= 6.0%, soybean chaff= 1.5%, milled corn= 55.3%, wheat chaff= 3.0%, powder molasses 2.8%, sewage sludge= 20,0%, ground soybean (grain)= 0.9%, vegetal oil= 5.0% and protein 3.4%. Sewage sludge (supplied by SABESP, Suzano, São Paulo State) was air dried and sieved to 2 mm It contained: dry matter= 94.2%, energy= 0.3 Mcal kg^{-1}, ether extract= 0.9%, mineral= 16.3%, crude protein= 0.78%, P= 141,3, K= 108.3, Ca= 150.0, Mg= 216.0, Cu= 9.3, Fe= 3941, Mn= 17.4, Zn= 8.4, Cr= 8,6, Cd= 0.3, Ni= 6.3, Pb= 9.6 (mg kg^{-1}), It also contained 1.6 10^5 spors of bacteria of the *Clostridium genus*. The two rations contained 18% of crude protein, 63% of total digest nutrients, Ca= 0.3% and P= 0.2%. Males of pure dutch race, 3-4 days old, were kept in mobile individual stalls and received water (free way), 2 kg day^{-1} ration and 0.5 kg day^{-1} alfalfa hay for 14.5 weeks, after what they were submitted to a jejune period of 48 h, weighed and started to receive the two types of rations (start of the experiment). The animals received 2.0 kg ration day^{-1} in the first 28 days and and 2,5 kg from 28 to 56 days. After 56 days, the supplying of sewage sludge was stopped and all the animals received the same ration. At 84 days the animals were transferred to collective stalls (one for each treatment) and started to receive an additional 8 kg day^{-1} of corn silage. Daily, the intake of ration was measured and each 28 days (0, 28, 56, 84, 112) the animals were weighed. At 56 days, blood samples were collected for hemogram and heavy metals analysis and muscle samples were collected for heavy metals quantification.

3. Results and Discussion

The intake of ration by the animals was not different till 28 days, but at 56 days, the intake of ration by the animals that received sewage sludge was lower (Figure 1).

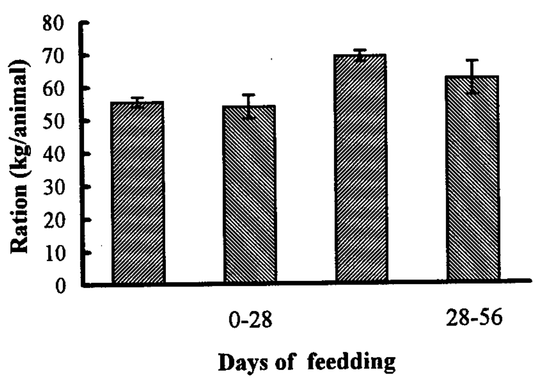

Figure 1. Intake of ration by animals receiving or not sewage sludge in the ration (left bar= no sewage sludge; right bar= with sewage sludge).

The weight gain till 28 days was not different in the two treatments, but at the 56 days the weight gain of the animals of the control treatment was about 2.5 fold that of the animals that received sewage sludge in the ration (Figure 2). After sewage sludge

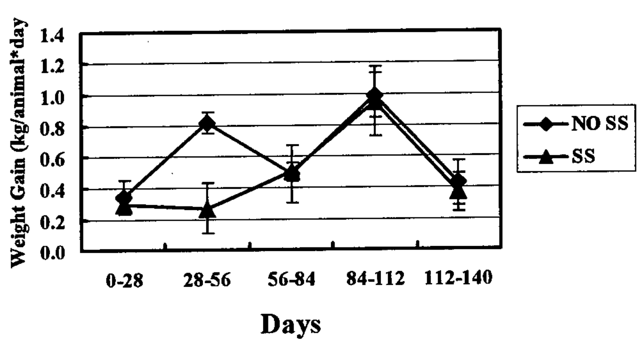

Figure 2. Weight gain of animals receiving ration containing or not sewage sludge (NOSS= without sewage sludge; SS= with sewage sludge).

was removed from the ration, the weight gain was similar in the two groups of animals. The large weight gain observed at 112 days is attributed to the type of ration and the decrease at 140 days corresponded to the suspension of the furnishing of concentrate and the supplying of elephant grass and sugar cane. The blood hemogram was not different in the two treatments, the same occurring in relation to the blood content of Zn and Fe. Cd, Cr, Pb, Cu and Ni were not detected in blood and muscle.

No heavy metals was founded in blood and muscle samples.

4. Conclusions

During receiving sewage sludge in the rations, the animals presented a decreasing in the weight gain, which was recuperated when the residue was removed from the ration. Heavy metals presented in sewage sludge was not found in blood and muscle.

Acknowledgement

The authors thanks CNPq and FAPESP, for the financial support, and SABESP, for the sewage sludge supplying.

EFFECT OF SEWAGE SLUDGE ASSOCIATED CD ON SORGHUM PLANTS

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1. Introduction

Population increasing and industrial development have caused an increasing of water use and pollution. Water waste treatment, in order to return it to the streams and rivers without causing environment pollution, has originated an accumulation of sewage sludge, which presents in its composition plant nutrients, so that its use in agriculture may solve two great problems: the correct disposal of the residue and a decreasing in the use of mineral fertilizers, which are also cause of pollution.

But, sewage sludge also contents in its composition heavy metals, among then the Cd, one of the most toxic metals for plants and animals. Thus, the objective of this work was to estimate the effect of sewage sludge, when added to the soil after enrichment with Cd, on sorghum plants growing under greenhouse conditions.

2. Material and Methods

Anaerobic sewage sludge (C= 20.3, N= 4.2, P= 2.8, K= 3.9, Ca= 3.5, Mg= 5.6, S= 5.8 g Kg^{-1}; Cu= 429, Fe= 26630, Mn= 285, Zn= 510, Cr= 132, Cd= 10, Ni= 238, Pb= 200 mg Kg^{-1}) was air dried, ground to 20 mesh and enriched with Cd, as cadmium chloride, to obtain 10, 15, 60, 240, 960 mg Cd Kg^{-1} sewage sludge). Soil sample (a Dark Yellow Latosol with pH_{CaCl_2}= 4.1, OM= 25 g Kg^{-1}, P_{resin}= 6, K= 1.2, Ca= 4.0, Mg= 3.0, H+Al= 58.9, $mmol_c$ dm^{-3}, V= 12%, sand= 750,silt= 70, clay= 180 g Kg^{-1}) was air dried, sieved to 2 mm, mixed to dolomite limestone (to obtain V= 70%) and placed into pots (diameter= 15 cm and high= 30 cm). The soil from the top 0-10 cm of each pot was then removed, mixed with sewage sludge at the rate 40 Mg ha^{-1} and returned to the same pot. Each pot received deionized water in order to obtain 70% of the WHC and was daily irrigated for 10 days, so the water content was kept near that value. In the 10th day after sludge incorporation, 5 seeds of sorghum (AG-300-tanino FROM Agroceres) were sowed in each pot. When the plants were 10 cm high, the excess of plaints was removed, keeping only 1 plant per pot. Eighty days after sowing, each pot received a NK fertilization. The experiment design was a totally randomized with 5 treatments (rates of Cd) and 4 replications. Plants were sampled at 130 days after sowing, split in root (0-10, 10-20 and 20-30 cm), stalk, leaves and grains, washed with 0.1 mol L^{-1} (1 mol L^{-1} for roots) HCl, diluted solution of detergent, water, distilled water and deionized water, dried at 70°C, weighed, ground to 40 mesh and analyzed for Cd content, after a digestion with a mixture of nitric+perchloric acids

3. Results and Discussion

Total dry matter production by sorghum plants increased with Cd enrichment till the dose 60 mg Kg^{-1} and decreased with the higher rates of the heavy metal. The same occurred for the different parts of the plant, except for stalk, whose higher dry matter production was observed in the rate 15 mg Kg^{-1} (Figure 1). These data let us to conclude that 60 mg Kg^{-1} of Cd in sewage sludge was bėnefit to sorghum plants production. The accumulation of Cd in the dry matter of sorghum plants increased linearly with the rates of the metal till 160 μg of Cd per plant (dry matter basis).

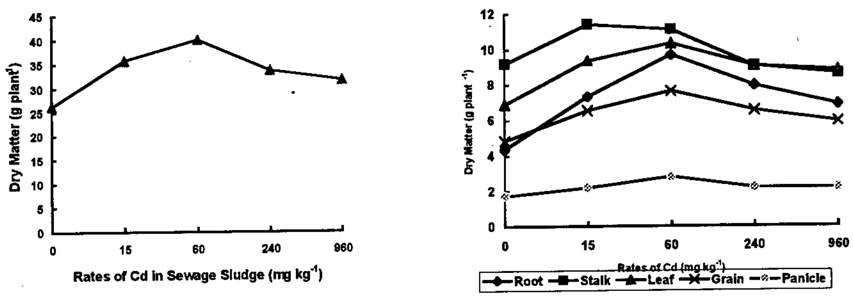

Figure 1. Dry matter production by sorghum plants cropped in a Dark Yellow Latosol treated with 40 Mg sewage sludge ha^{-1} enriched with increasing rates of Cd.

The distribution of the metal in the parts of the plant was stalk (except for the rate 960 mg Kg-1, when the content of the element was higher in the roots 0-10 cm depth)> root> stalk> grains. Considering the distribution of the Cd in the root, it was observed the following sequence: 0-10 cm>10-20 cm>20-30 cm (Figure 2).

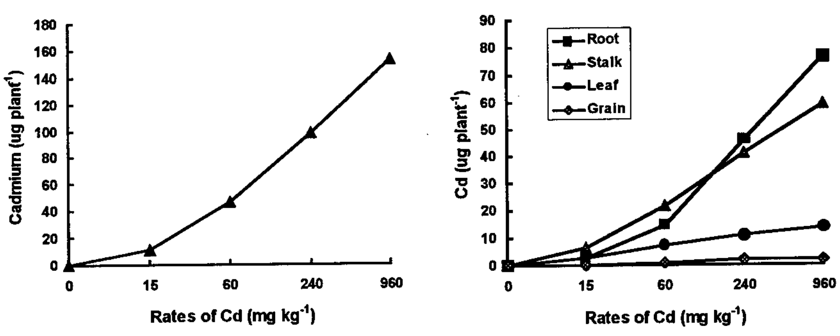

Figure 2. Accumulatioṅ of Cd in sorghum plants cropped in a Dark Yellow Latosol treated wuth 40 Mg sewage sludge há$^{-1}$ enriched with uncreasing rates of Cd.

4. Acknowledgements

The authors are grateful to FAPESP, for the financial support, and to SABESP, for supplying the sewage sludge.

DETERMINATION OF THE BINDING CHARACTERISTICS OF ZN IN SOILS AMENDED WITH BIOSOLIDS

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1. Introduction

The disposal of biosolids to agricultural land is, in the short-term, an economically and environmentally acceptable method of recycling essential nutrients to the soil. However, there are risks associated with the use of biosolids and in particular these are related to the presence of potentially toxic elements (PTE) within the biosolid. The ecological and toxicological impacts of metals in soils are directly related to their partioning between mobile and immobile species. Many factors affect the degree and strength of metal complexation in soils and subsequently their bioavailability within the agroecosystem.
The objectives of this study were to determine the binding characteristics for Zn in an agricultural soil amended with municipal biosolids. The experiments involved batch adsorption and equilibrium dialysis titration of soil organic components. Of the PTE within biosolids, Zn is arguably of greatest concern because it is often present in high concentrations in the biosolids and is relatively labile in soil.

2. Materials and Methods

A freely draining argillic brown earth in southern England was amended with a single application of municipal biosolids at rates equivalent to 0, 5, 7.5, 10, 15 and 20 t ha^{-1}dry solids. Winter wheat was then grown on the field plots and after one year the soils were sampled.
For quilibrium dialysis, a dialysis bag (<3000 Da) containing a weak salt solution was placed in a volume of soil organic matter extract (0.1M $Na_4P_2O_7$, 80:1 soil:solution), metal ions are then titrated into the outer solution. The diffusate from the titration contains only 'free' Zn ions (< 3000 Da) while the retentate contains complexed Zn species (> 3000 Da) and 'free' Zn ions (KARAPANAGIOTIS et al., 1991). From a plot of the ratio of the Zn concentrations in the diffusate/retentate ('free' Zn/complexed Zn) against the concentration of Zn in the diffusate ('free' Zn) it is possible to determine the organic complexation capacity (L_T) and the conditional stability constant (the equilibrium constant of a reaction forming a chelate) for the organo-Zn complexes formed (K). Batch adsorption experiments were carried out on the soils and the sorption data were fitted to the linearized Freundlich equation. The Maximum Likelihood Program (ROSS, 1987) was used to perform a parallel curve analysis on the straight lines produced from the plots of the data from the equilibrium dialysis titrations and the isotherms.

3. Results and Discussion

The maximum concentration of 'total' Zn observed in the field trial was at the greatest biosolid application rate (Table 1). However, this value was within the 'normal' range for soils in England and Wales, and was considerably lower than current UK regulation limits of 200 mg Zn kg^{-1} for a soil of this pH value (MAFF, 1993).
There was a significant increase in organic carbon content of the amended soils (Table 1). Therefore, we may have expected a significant increase in the soils affinity for Zn (KARAPANAGIOTIS et al., 1991). The application of biosolids to agricultural land arguably

alters the characteristics of the organo-metal complexes within the soils, which may subsequently affect the bioavailability.

Table 1: Selected physico-chemical characteristics of biosolids and amended soils (means±1S.E).

	Zn mg kg^{-1}	pH ($CaCl_2$)	Total C, %.	Total P, $mgkg^{-1}$	Zn in $Na_4P_2O_7$ Extract mg kg^{-1}
Biosolid	610±21.7	5.33±0.01	-	-	
Control Soil - 0 t/ha	26.3±0.8	5.11±0.05	2.54±0.11	241.6±21.5	3.645 ± 0.086
5 t/ha	27.2±1.4**	5.06±0.05*	2.62±0.05*	310.5±21.6**	5.235 ± 0.188**
7.5 t/ha	27.3±0.7**	4.96±0.09*	2.61±0.07*	338.6±38.3**	5.340 ± 0.208**
10 t/ha	28.3±0.4**	4.93±0.07*	2.59±0.05*	343.3±54.6**	5.828 ± 0.208**
15 t/ha	30.0±0.7**	5.10±0.03*	2.70±0.08*	389.9±31.9**	5.863 ± 0.296**
20 t/ha	34.3±1.1**	5.05±0.09*	2.77±0.10*	471.2±63.8**	7.941 ± 0.207**

Table 2: The Freundlich parameters from the sorption plots and the complexation capacities and stability constants from the dialysis titration of the soil organic component.

	Sorption data for Zn		Conditional stability constants of organo-Zn complex (logK')	Complexation capacities of soil organic component (L_T mol g^{-1} of OM)
	1/n	log Kf.		
0 t/ha	0.962	1.582	6.57	9.25
5 t/ha	1.180	1.454**	5.51*	0.97
7.5 t/ha	1.212	1.428**	6.47*	3.48
10 t/ha	1.112	1.496*	6.54**	3.98
15 t/ha	1.102	1.481	6.55**	4.78
20 t/ha	1.046	1.445*	6.55*	6.72

*Significant different from control at 5% level.
**Significant different from control at 1% level.
log Kf = Freundlich distribution coefficient and is equal to the intercept from the isotherm and is a measure of the partitioning of the metal between solid and liquid phases.
1/n = slope of the straight line is a measure of the affinity of the soil for the metal.

Despite the potential affinity of components of the organic fraction for Zn there was no corresponding increase in organic retention characteristics for Zn (Table 2). Indeed, while no change was noted in the complexation capacity of the organic component in the amended soils there was a significant decrease in the strength of the stability constants of organo-Zn complexes formed.

The sorption data from the batch equilibration experiments in Table 2 shows a significant decrease in the Freundlich distribution coefficient for Zn compared with the control, most likely due to the presence of Zn as 'free' metal ions in solution or possibly low molecular weight (<3000 Da) complexes.

4. Conclusions

- Equilibrium dialysis titration allows trends in the organic retention of metals to be determined experimentally despite the relatively ill-defined nature of soil organic matter.
- Addition of Zn to soils amended with biosolids results in a corresponding decrease in the organic binding strength of the Zn complexes formed and overall soil sorption properties.
- This may increase the potential bioavailability and ecotoxicology of Zn in the agroecosystem even at the low biosolids application rates.

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REDUCING THE HEAVY METALS TOXICITY IN SLUDGE AMENDED SOIL USING VA MYCORRHIZAE

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1.Introduction

Utilization of sewage sludge on agricultural land increases the concentration of heavy metals in soil. Studies suggest that leguminous crops dependent on symbiotic N_2- fixation may be sensitive to the toxic effects of heavy metals present in sludge. In soil newly amended with sludge, Ham and Dowdy (1978) reported increased uptake of Zn and Cd, decreased nodulation, but improved soybean yield up to 2 years after the initial application. Little attention has been given to the role of vesicular-arbuscular mycorrhizae (VAM) in environments containing elevated and potentially toxic level of mineral elements, though inoculation with VA mycorrhizae reduced Zn and Cd concentration in plant leaves grown in soil with high concentrations of these metals.

2.Materials and Methods

A pot experiment was carried out in the greenhouse of the National Research Center, Dokki, Cairo, Egypt. Sandy soil from Ismalillia and sewage sludge from two different sewage treatment plants at Abou-Rawash and Al-Gabal Al-Asfar were used.

Soil was packed in earthenware pots 30 cm in diameter and 40 cm in depth. Air dried sewage sludge from each source was added to soil at rates of 0, 0.5, 1.0, 2.0 and 4.0%. Half of the pots of each treatment were inoculated with mixed VA mycorrhizal spores (600 spores of *Glomus clarum; Glomus mosseae* and *Glomus fasciculatum* per pot). Five seeds of faba bean (*Viceae faba)* variety Giza 2, were sown into each pot and the pots were watered as needed.

3.Results and Discussion

Application of sewage sludge up to 2% increased faba bean growth, nodule number and weight, and phosphorus and nitrogen contents (Table 1). Sewage sludge treated soil where the heavy metals were present in high concentrations, inoculation with VA mycorrhizae reduced the concentration of heavy metals (Table 2).

Table (1): Effect of sewage sludge application and VA mycorrhizae on nodulation, shoots dryweight, nitrogen and phosphorus contents of faba bean plants .

Treatments	Nodules No./ plant		Nodules dry weight (mg/plant)		Mycrrhizal root infection %		Shoots dry weight (g/plant)		N-Content mg/plant		P-content mg/plant	
	- VAM	+ VAM	- VAM	+ VAM	- VAM	+ VAM	- VAM	+ VAM	- VAM	+ VAM	- VAM	+ VAM
0.0%	36	39	153	160	12	64	7.15	7.62	150	178	14.3	17.5
0.5%	37	42	151	182	11	60	7.24	7.73	155	177	18.1	23.2
2.0%	54	60	240	273	12	60	9.74	11.35	253	295	34.1	45.4

Table (2): Effect of sewage sludge application and inoculation with VA mycorrhizae on the concentration of heavy metals in faba bean shoots (μg/g).

Treatments	Mn		Zn		Cu	
	- VAM	+ VAM	- VAM	+ VAM	- VAM	+ VAM
0.0%	16.1	20.9	19.0	22.8	3.22	4.72
0.5%	72.9	47.5	43.0	32.2	8.84	7.36
2.0%	55.2	41.2	53.7	35.4	7.45	6.91

4.Conclusions

The role of VA mycorrhizae in reducing the hazardous effect of heavy metals when present in high levels in the media of growing plants.

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METALS IN LEGUMES GROWN ON SOIL AMENDED WITH URBAN SEWAGE SLUDGE IN THE FIELD UNDER SEMI-ARID CLIMATIC CONDITIONS

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1. Introduction

The development of the treatment of wastewater in Tunisia has led to a production of approximately 1 300 000 m^3 of liquid sewage sludge per year. Fifty percent of the sludge is disposed of on agriculture land, in replacement of the lacking farmyard manure for conservation of soil fertility. In general, sludge is applied to soils used for legume production and orchards at rates similar to those used for farmyard manure.

To determine whether the application of sewage sludge has an influence on the quality of legumes, an experiment was conducted in the field, and the metal content of various plant organs including edible parts was measured.

2. Materials and Methods

The field experiment was set up in 1994 at the Nabeul experimental station of INRGREF in North-Eastern Tunisia, an area characterised by semi-arid climatic conditions. Soil was a sandy-loam soil developed on alluvial deposits. It was low in organic matter and had a pH of 7.5. Sludge was collected at the SE1 Hammamet treatment plant. It was an aerobically digested sludge dehydrated on drying beds. Total heavy metals (Cd, Co, Cu, Fe, Mn, Pb and Zn) were determined by atomic absorption spectrophotometry after diacid digestion (HF - $HClO_4$) of the sludge. Characteristics are presented in table 1.

Table 1: Characteristics of sewage sludge: N and C (in % DM) and total metal concentrations (in mg kg^{-1} DM except Fe expressed in g kg^{-1} DM)

		% DM		mg kg^{-1} DM						
DM	pH	C	N	Cd	Co	Cu	Fe	Mn	Pb	Zn
47%	6.7	36.8	3.1	5	17.7	212	9.4‰	108	449	510

DM: dry matter

Sludge was applied at four rates, i.e. 0, 20, 40, and 60 t DM per ha on 50 m^2 plots. Six plants were grown, i.e. pepper (*Capsicum annuum*), chard (*Beta vulgaris* L.), carrots (*Daucus carota* L.), radish (*Raphanus sativus* L.), tomato (*Solanum lycopersicum*), and lettuce (*Lactuca sativa*), each on a separate set of plots. Treatments were repeated five times, hence the experience exhibited a total number of 120 plots. Two successive crops were conducted preceded by a sludge application. Plant growth was conducted according to classical practices, e.g. irrigation, fertilisation and harvest. Harvest occurred either at the end of (lettuce, radish, carrot) or during (pepper, chard) the crop cycle. At the end of each crop, organs were separated, e.g. root, stem, leaf, and fruit, and analysed separately for metals (Cd, Co, Cu, Fe, Mn, and Zn) by flame atomic absorption spectrophotometry after acid mineralization. Data were analysed with the statistical programme STATITCF.

3. Results and Discussion

Results show that metal content in edible plant parts collected on control plots is different according to the species. For cadmium, values vary from 0.25 to 5.5 mg kg^{-1} dry matter, and decrease with the following order tomato>chard>radish>pepper>lettuce=carrots. Sharp differences are also observed according to the plant organ.

Applications of sewage sludge modified the content in cadmium, cobalt, copper and zinc in plant tissues. At the loading rate of 20 t DM ha^{-1} a significant increase was recorded for zinc in all plant species tested, and response to sludge application was different according to the organ considered. Cadmium content was increased in edible plant parts of all plant species. Increase in cadmium reached 5.5 mg/kg DW in tomato. Other loading rates produced higher metal concentration in plant tissues.

From one crop to another, and, for a given crop, from one harvest to another (e.g. pepper, chard) a large range of variation was observed in metal content for all elements and plant tested.

4. Conclusions

The use of sewage sludge for legume production may induce an increase in concentration of metals, either essential or not, in plant tissues and especially in edible plant parts. However, loading rates used in this field experiment were higher than those applied by farmers. Moreover, this experiment, which was conducted under natural conditions and with regular farming practices, showed a wide range of variation due to sludge application, much lower than that observed between two following harvests or crops. Hence, risk of contamination of legumes by metals following sludge application should be rather low. Nevertheless, soil quality should be frequently controlled, and sludge quality should be constantly improved to ensure the quality of legumes on the long-term.

EVALUATION OF HEAVY METAL POLLUTION ON BARLEY CROP BY AGRICULTURAL USE OF DIFFERENT BIOSOLIDS

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1. Introduction

Soil application of biosolids is important because of the increased production of wastes and the disappearance of other possible disposal sites (Hall, 1995). The properties of biosolids organic matter affect the fertility and potential production of soils (Clapp et al., 1986). However, heavy metals introduced into soils must be observed to assess the possibility of transfer to the food chain (Chang et al, 1987).

2. Materials and Methods

The experiment was conducted on a silty-clay soil (Madrid, Spain), in which dewatered (D) and composted (C) biosolids were applied at rates of 20 and 80 t ha^{-1}. The field study was undertaken on 15x3 m^2 plots cropped with barley and the design was a randomised complete block with three replicates. Biosolids were incorporated into the first 15 cm of soil and then Beka barley was planted in December. Plants were harvested at maturity in June and heavy metal contents (Mn, Zn, Cu, Cd, Ni, Cr and Pb) were analyzed, both in grain and straw.

Table 1. Chemical analysis of soil and biosolids (*)

					mg kg^{-1}						
	pH	E.C. †	C org ‡	N ‡	Mn	Zn	Cu	Pb	Cd	Cr	Ni
Soil	8.1	0.9	10.1	1.0	125	32	10	5	0.1	6	6
D	5.9	6.7	225	50	111	486	127	145	15	450	13
C	7.0	5.7	156	13	227	882	145	209	7	702	33

(*) dry matter. † mS cm^{-1}. ‡ g kg^{-1}

Soil pH was measured on 1:2.5 soil:water and 1:5 sludge:water ratio. Electrical conductivity was measured on saturated paste of soils and on 1:5 sludge:water ratio. Total organic carbon was analyzed by dichromate oxidation and titration with ferrous ammonium sulphate. Total nitrogen was determined by the Kjeldahl method. For the total content of heavy metals, samples were digested with nitric and perchloric acids and then analyzed by atomic absorption spectrophotometry. All data were analyzed using the one way analysis of variance (ANOVA) and means were compared using the Tukey's test (95% of significance).

3. Results and Discussion

The analysis of heavy metals both in grain and straw demonstrates the absence of pollution or toxic levels (Chang et al., 1982) even in high doses. Highest values (mg kg^{-1}) of these elements obtained in this experiment are: in grain Cd (<0.1), Cr (<4.0), Pb (<5.0) and Ni (<1.0), in straw Cd (<0.1), Cr (<6.0), Pb (<0.6) and Ni (<4.0).

Table 2. Micronutrient and heavy metal contents in barley plants (mg kg^{-1})

	Manganese		Zinc		Copper	
	grain	straw	grain	straw	grain	straw
	11 a	11 a	24 a	14 a	5 ab	4 a
C-20	11 a	13 ab	41 b	31 b	6 bc	4 a
C-80	12 a	19 b	55 c	52 c	7 c	6 a
D-20	11 a	16 ab	35 b	23 ab	5 ab	4 a
D-80	11 a	13 ab	36 b	20 ab	5 ab	3 a

In each column values followed by the same letter are not significant at $p \leq 0.05$.

The application of biosolids did not significantly increase manganese and copper in barley plants; only an increase in comparison to the control was observed for copper in the grain of the plots amended with compost (table 2). This material also produced a significant increase in the content of zinc in both barley grain and straw. However, values obtained for these elements in barley plants are in the same range of the ones reported by other authors for similar experiments (Chang et al., 1982).

4. Conclusions

A field experiment was conducted to study the effect of two different biosolids (applied at two rates) on the heavy metal content of barley plants. No contamination was observed either in grain or straw; however, higher values were found with the application of high-rate composted biosolids.

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METAL SOLUBILITY IN A BIOSOLID AMENDED SOIL

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1. Introduction

Toxic element mobility in biosolid amended soils is a concern because of the possibility of groundwater and food supply contamination. The results of a batch equilibrium desorption study on sewage sludge amended soils is presented.

2. Materials and Methods

Soil samples were taken from research plots at the Elora Research Station (Ontario, Canada), to which sewage sludge had been applied between 1972-1980. The soil on which the plots are situated is a Conestogo loam (Gleyed Melanic Brunisol, Aquic Eutrochrept). To the plots were applied three different sludges (a Ca-sludge, a Al-sludge and a Fe-sludge) at three application rates in a completely randomized experimental design. A control, to which only N fertilizers were applied, was also included. For the purposes of this study, only the highest application rates (1600 kg N/ha) for each of the three different sludges plus a control were sampled.

A batch equilibrium experiment, (over a pH range of 3-10, in 0.01 M $LiClO_4$, two replications) was performed. The samples were analyzed for sulfate, chloride and metals using ICP-MS. Total organic carbon was also determined. Additionally, the amount of crystalline and amorphous Fe and Mn in each soil was determined using sodium dithionite-ammonium oxalate extraction. The results were graphed and modeled using the computer model, MINTEQ.

3. Results and Discussion

Metal concentrations ranged from a low of about 0.5 $\mu mol\ L^{-1}$ for V (at pH = 8.5) to a high of approximately 350 $\mu mol\ L^{-1}$ for Zn (at pH = 4). Concentrations of soluble cationic metals, such as Cd and Zn, increased with increasing pH. Anionic metals, such as V, demonstrated the opposite behavior: soluble concentrations increased with increasing pH values.

To understand the behavior of metals in biosolid amended soils it is necessary to consider adsorption reactions onto mineral surfaces, complexation by soil organic matter and the formation of insoluble precipitates (EVANS et al., 1995). The observed trends are probably due to variable charge surfaces associated with the oxides and (oxy) hydroxides of Fe, Al, and Mn. At pH values below the point of net zero proton charge (PNZPC) the surfaces or edges are positively charged. At the PNZPC, the surfaces or edges are neutral, whereas at pH values above the PNZPC, they are negatively charged. Specific adsorption of cationic metals involves the formation of an inner sphere complex with the negatively charged site. Thus, specific adsorption of cations increases as pH increases, reaching a maximum at a pH about 3 units below the first hydrolysis constant of the metal (EVANS et al., 1995). The specific adsorption of anions also involves the formation of an inner sphere complex, this time with the positively charged site. Since the concentration of the dissociated anion increases as pH increases, while the number of positively charged sites decreases as pH increases, the amount of adsorption increases to a maximum at or near the pKa of the weak acid or oxyanion and then decreases (EVANS et al., 1995).

Cationic metals known to form strong carbonate complexes, such as Cd and Cu, showed an

additional phenomenon - soluble metal concentrations increased at pH levels above about 7.5.
The results were modeled using the computer model, MINTEQ. A constant capacitance model was used to calculate adsorption onto variable charged sites. Aqueous speciation was modeled from experimental determination of pH and the concentrations of ligands such as Cl^-, and SO_4^{2-}. Since the maximum concentration of any element in solution is controlled by the solubility of precipitates that contain that element, precipitation/ dissolution of the important precipitates (hydroxides, carbonates, and sulfides) was incorporated into the model. Finally, organic matter can complex with metals, constituting an important sink for metals in biosolid amended soils. Complexation of metals with humic material may be represented by a reaction between a metal and a diprotic acid, thus total soluble organic carbon was used to estimate the content of diprotic acid in the soil solution.
Modeling of desorption curves resulted in underestimates of cationic metal adsorption, indicating the presence of an adsorbing surface not considered in the model.

4. Conclusions
The adsorption of metals onto biosolid amended soils is highly pH dependent. In addition, the desorption of some cationic metals which form strong carbonate complexes at high alkalinity gives reason for concern about the application of biosolids onto alkaline soils. This illustrates the need to develop guidelines that establish an upper limit of pH at which biosolids may be applied.
This study illustrates that modeling of soil chemical processes is an important tool both for predicting the bioavailability of metals in biosolid amended soils and for investigating the soil chemical processes occurring in these soils.

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UPTAKE AND ACCUMULATION OF ZINC FROM SEWAGE SLUDGE AMENDED SOIL

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1. Introduction

Land application provides a more sensible way for the disposal of sewage sludge by reutilizing its enriched nutrients and organic matter content (Smith, 1996). However, sewage sludge produced in Hong Kong is originated both from domestic and industrial sewage effluents and contains relatively high heavy metal contents (Wong and Wong, 1998). Therefore, it is the aim of the present study to assess the uptake and accumulation of Zn from sewage sludge by *Cynodon dactylon*.

2. Materials and Methods

Sewage sludge was collected from the Tai Po and Yuen Long wastewater treatment plant. An acidic loamy soil, collected from Produce Green Foundation Co. Ltd., was amended separately with sewage sludge generated from the Tai Po and Yuen Long sewage treatment plant at application rates of 0, 0.25, 0.5, 1 and 4% (w/w dry weight basis). DTPA-extractable Zn contents were determined on sewage sludge amended soils while chemical speciation of Zn was determined on the sludge and soil samples (Page et al., 1982; Tessier et al., 1980). 100 seeds of *C. dactylon* were sown for each pot and plants were allowed to grow for a period of 10 weeks. An additional acidic loamy soil only control receiving a complete fertilizer only was also prepared for comparison. After harvesting, the dry weight yields of shoot and root were determined, and plant tissue were digested by di-acid digestion method followed by Zn measurement using atomic absorption spectrometry (Page et al., 1982).

3. Results and Discussion

The residual and Fe-Mn oxides fraction accounted for more than 50% of Zn in sewage sludge which is in line with the results of others (McLean and Dekker, 1978). The Zn content in the organic fraction did not differ significantly between the soil and sewage sludge. The exchangeable and carbonate fraction was about 20-30% of total content of Zn in sewage sludge, and was significantly higher than that of the soil. DTPA extractable Zn contents of sewage sludge were higher than that of the loamy soil. Total Zn content of Yuen Long sludge were significantly higher than that of Tai Po sludge, probably due to the discharge from tannery and electroplating factories of the nearby Yuen Long Industrial Estate. Addition of both sludges significantly increased the yield of *C. dactylon* at an application rate of <4% and the highest yield at 0.5% Tai Po sludge amended soil was similar to that of the control with fertilizer treatment. For both sludges, especially Yuen Long sludge, Zn contents in shoot tissue increased significantly according to the rate of sludge application. Zinc contents in shoot tissue grown in Yuen Long sludge amended soil were all higher than the phytotoxicity level of 350 mg kg^{-1}. This might explain the lower yield for *C. dactylon* grown in Yuen Long sludge amended soil than that of Tai Po sludge.

4. Conclusions

In summary, Yuen Long sludge exerted an adverse effect on plant growth due to the high Zn content in the sludge as compared to Tai Po sluge. Hence, Tai Po sludge is recommended to be a potential organic amendment for poor quality landfill soil similar to those used in the present study at an application rate of <4% for the growth of *C. dactylon*.

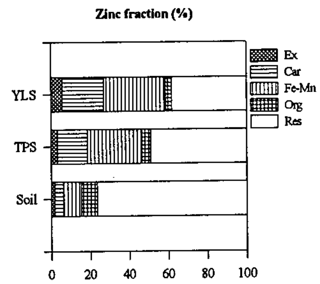

Figure 1. The percentage of various chemical forms of Zn in loamy soil, Tai Po sludge (TPS) and Yuen Long sludge (YLS).

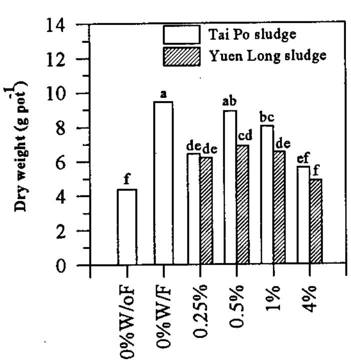

Figure 2. Dry weight yield of *C. dactylon* in soil amended with 0.25, 0.5, 1 and 4% (w/w) of TaiPo and Yuen Long sludge, and in soil with or without fertilizer treatment.

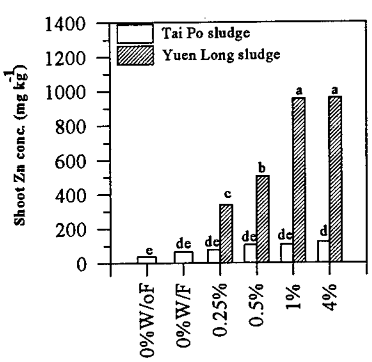

Figure3. Zinc contents of plant shoot of *C. dactylon* grown in loamy soil mixed with 0, 0.25, 0.5, 1 and 4%(w/w) Tai Po and Yuen Long sludge, and in soil with or without fertilizer treatment.

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SEWAGE SLUDGE COMPOST AS A GROWTH MEDIUM FOR TOMATOES

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1. Introduction

There is an ever-increasing demand for peat and peat-based mixtures for greenhouse crop production. Composts made from municipal and industrial sludges and other solid wastes can be effectively substituted for peat if they pose little risk to human or animal health or to the environment. Furthermore, these waste materials may become economically viable composts by reducing the need and cost to landfill the feedstocks. Various composts have been used in tomato production both in the field and the glasshouse (Sterrett et al., 1983; Stilwell, 1993; Ozores-Hampton et al., 1994; Campbell et al., 1995; Maynard, 1995; Madrid et al., 1998). Some experiments have evaluated MSW compost while others have used sewage sludge. Fruit yield and fruit and tissue elemental analysis has been employed to evaluate the suitability of the waste composts to replace peat. Therefore, an experiment was conducted to determine whether sewage sludge compost, alone or in combination with peat, would make an effective potting medium for greenhouse tomatoes. Fruit yields, leaf nutrient and leaf metal contents were compared in plants grown in peat and sewage sludge compost.

2. Materials and Methods

Four treatments consisted of 100% compost, a 50%-50% (v/v) peat-compost mixture, with (F) and without (NF) added fertilizer, and 100% peat. Except for the NF mix treatment, the media were fertigated with soluble 20-20-20 (N-P-K) fertilizer bi-weekly. The compost used in this experiment was a composite of four types of sewage sludge-racetrack manure compost (described in Warman and Termeer, 1996) aged six months longer than the composts evaluated in Warman and Termeer (1996). Three tomato seedlings (Lycopersicon esculentum Vendor) were transplanted into boxes measuring 75 cm (length) * 25 cm (width) * 20 cm (depth) filled with one of the three media. Treatments were replicated four times and arranged in a Latin Square design in a naturally-lighted glasshouse. Plant leaf samples were taken between the first and second flower cluster from the top when the first fruit formed. Fruit was picked and weighed when ripe. Washed and dried tissue was ground and digested with nitric acid then analyzed for 6 essential nutrients (except N) and 8 metals by ICAP; N was analyzed from a sulfuric-peroxide digest. After leaf samples were taken, some plants showed symptoms of blossom end rot (Ca deficiency); therefore, all the treatments were supplemented with an equal amount of dolomitic lime at 8 Mg/ha.

3. Results and Discussion

There was a high degree of variability in the nutrient and metal content of the leaf tissue and, therefore, many of the differences between treatments were not significant. The 100% compost media produced the highest fruit yields/plant and the highest N content in the leaves, which differs from the Swiss chard experiment in Warman and Termeer (1996) where the 100% compost treatment performed poorly, but the 67% compost-33% peat was the best growth media. The NF peat-compost mix produced a higher tissue P content compared with the other treatments, which is likely associated with a growth x tissue concentration factor; however, this

can not be proven since plant dry matter yields were not measured. The 100% peat produced significantly lower leaf tissue Ca compared with the NF peat-compost media. The lime addition after sampling somewhat corrected this Ca problem. Correspondingly, the peat media produced also produced significantly higher leaf Cu, Zn, and Cd compared to the peat and compost (excluding Cu). Leaf K, Mg, S, B, Fe, Cr, Pb and Ni were not influenced by the high tissue Mn, possibly related to the low Ca and pH of the peat. The NF peat-compost treatment treatments.

4.Conclusions

The results showed that 100% sewage sludge compost medium did not increase the metal content of tomato leaf tissue, although an unfertilized mix of peat and compost produced higher leaf Mn, Cu, Zn and Cd compared with either the compost or peat treatments, or both.

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Table 1. Tomato Fresh Weight Yield (kg/plant) & Leaf N, P, K, Ca, Mg & S Content (mg/g)

Growth Media	Yield	N	P	K	Ca	Mg	S
compost	2.70	33.5	9.0	48.8	24.4	5.8	9.6
peat	1.48	29.3	10.1	41.1	18.7	6.2	9.5
F peat-compost	2.13	25.9	12.1	44.8	25.3	5.6	10.6
NF peat-compost	1.53	24.7	15.1	41.6	25.3	6.3	10.3
MSE	2.04	9.35	4.40	64.65	3.51	0.39	1.23
LSD	2.47	5.3	3.6	13.9	8.0	1.1	1.9

Table 2. Fe, Mn, Cu, Zn, and B Content of Tomato Leaf Tissue (mg/kg).

Growth Media	Fe	Mn	Cu	Zn	B
compost	116	260	16	51	56
peat	114	494	11	46	56
F peat-compost	99	381	17	68	54
NF peat-com	59	654	22	91	69
MSE	1896	32787	19	369	621
LSD	75	313	7.5	33	43

Table 3. Cd, Cr, Pb and Ni Content of Tomato Leaf Tissue (mg/kg)

Growth Media	Cd	Cr	Pb	Ni
compost	0.3	0.8	0.5	2.7
peat	0.5	1.0	0.4	1.4
F peat-compost	0.7	1.1	0.3	1.4
NF peat-compost	1.3	1.0	0.3	1.4
MSE	0.11	0.21	0.08	2.0
LSD	0.6	0.8	0.5	2.5

Kinetics and Mechanism of Metal Sorption/ Release on Natural Material

(Special Symposium 9)

EXAFS STUDY OF MERCURY BONDING TO GOETHITE WITH ADSORBED HUMIC ACID

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1. Introduction

A number of studies demonstrated that surface-bound organic matter affects the adsorption of heavy metals on oxide mineral surfaces. For example, Tipping et al. (1983) suggested that enhanced Cu(II) sorption to goethite in the presence of adsorbed humic substances resulted from the creation of additional high-affinity binding sites. Metal binding in organo-mineral systems such as those found in soils apparently depends on the relative affinities of the metal for the inorganic and organic binding sites, the nature of interaction between the organic matter and the mineral surface, and chemical factors such as pH. We used extended X-ray absorption fine structure (EXAFS) spectroscopy to determine the local molecular bonding structure of Hg(II) in humic acid (HA), goethite, or goethite with adsorbed HA (goethite-humate complex).

2. Materials and Methods

Sulfur-rich HA was extracted from surface soil samples from a coastal marine wetland using a modification of the International Humic Substances Society procedure. For the goethite-humate complex, aqueous HA was adsorbed on goethite at pH 5.6 by equilibrating a suspension containing 30 g humic acid/kg goethite, and removing excess HA by repeatedly washing the complex with 0.1 M $NaNO_3$ at pH 5.6. The final sample containing 20 g humic acid/kg goethite was equilibrated with $HgNO_3$ (20 mmol Hg/kg goethite) at pH 5.6 and 0.1 M $NaNO_3$ background. The Hg-goethite-humate complex was concentrated for EXAFS analysis by centrifugation. Samples of Hg(II) bound to either goethite or humic acid were prepared analogously. Ratios of organic S-to-Hg were 0.6 for the humic acid sample and 0.7 for the goethite-humate complex.

EXAFS data acquisition was done at Beamline X-11A at the National Synchrotron Light Source, Brookhaven National Laboratory, Upton, New York. Data were collected at the Hg L_{III}-edge in fluorescence mode using a multielement, solid-state Ge detector (Sayers and Bunker, 1998).

3. Results and Discussion

Table 1 gives EXAFS fitting results for the bonding of Hg(II) in samples of the single-component sorbents and the goethite-humate complex. The local molecular bonding of Hg(II) in the complex most closely resembled Hg(II) in HA. First-shell bonding of Hg(II) in humic acid was primarily to oxygen, with about 20% Hg-S bonding determined from relative coordination numbers of Hg-O and Hg-S. EXAFS fitting also showed about 35% Hg-S bonding in the goethite-humate complex (Table 1). Fitting results for Hg(II) on goethite showed first-shell Hg-O bonding at 2.06 Å, with higher-shell Fe at 3.17 Å. Although the first-shell Hg-O bond lengths were comparable for all samples, no higher-shell Fe was found in the EXAFS spectrum for Hg(II) in the goethite-humate complex. The

combination of Hg-S bonding and a lack of higher-shell Fe indicated that Hg(II) in the goethite-humate complex was primarily bonded with the HA phase.

Table 1. EXAFS fitting results for Hg(II) bound to humic acid, goethite, or a complex consisting of humic acid adsorbed to goethite.

Sample	Bond Type	Coordination number†	Radial distance (Å)	Debye-Waller factor ($Å^2$)
Hg(II) on humic acid (S:Hg = 0.6)	Hg-O	0.9 ± 0.2	2.01 ± 0.02	0.0020§
	Hg-S	0.5 ± 0.1	2.34 ± 0.02	0.0023§
Hg(II) on goethite	Hg-O	1.4 ± 0.2	2.06 ± 0.02	0.0034
	Hg-Fe	0.5 ± 0.3	3.17 ± 0.02	0.0063
Hg(II) on goethite-humate complex (S:Hg = 0.7)	Hg-O	1.2 ± 0.2	2.00 ± 0.02	0.0020§
	Hg-S	0.7 ± 0.1	2.34 ± 0.02	0.0023§

† Coordination numbers for Hg-O bonding are normalized to red HgO, which is 2-fold coordinated in the near shell, and Hg-S bonding is normalized to tetrahedrally-coordinated HgS (metacinnabar).

§ Debye-Waller factors were fixed during fitting.

4. Conclusions

EXAFS spectroscopy data for Hg(II) bound to a goethite-humate complex showed an average local molecular bonding environment similar to that of Hg(II) on humic acid. The results indicated that Hg(II) in the complex was primarily bonded with organic functional groups.

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Acknowledgments

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ADSORPTION MECHANISMS OF PB ON AMORPHOUS SILICA: AN XAS STUDY

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1. Introduction

To accurately predict the fate and mobility of heavy metals in soil and aquatic systems, fundamental knowledge of the mechanisms involved in the adsorption of heavy metals by soils and soil constituents is required. In this study we focused on characterizing the sorption behavior and mechanisms of Pb on amorphous silica using X-ray absorption spectroscopy (XAS). Amorphous silica is a commonly occurring natural sorbent, and Pb is a toxic and often encountered pollutant in the environment. XAS is a valuable tool in studying metal reactions on mineral surfaces, since it provides molecular scale information on the bonding environment of the sorbed metal.

2. Materials and Methods

Pb sorption on silica was studied as a function of pH. The pH range used was pH 3.4-6.5. The BET surface area of the silica used was 280 $m^2\ g^{-1}$. The studies were conducted in a 0.1M $NaNO_3$ background electrolyte. The solids concentration was 3 g L^{-1}, and the initial Pb concentration was 3 mM. All studies were carried out in a N_2-purged glove box to prevent $PbCO_3$ precipitation. The silica was hydrated in background electrolyte for 24 hours prior to reaction with Pb. An appropriate amount of Pb stock solution was then added stepwise to achieve an initial Pb concentration of 3 mM. Different amounts of acid (1M HNO_3) or base (1M NaOH) were added to achieve the desired pH. The suspensions were allowed to react for 24 hours. The final pH was then measured in suspension, and the samples were centrifuged. The supernatants were passed through a 0.2 :m filter, acidified, and analyzed for Pb using AA spectrometry. The amount of Pb adsorbed was calculated from the difference between the initial and final Pb concentrations. The wet pastes of the samples with pH>4.89 were analyzed by XAS.

XAS spectra were collected on beamline X-11A of the NSLS at BNL, Upton, NY. A Si(III) crystal was used to scan the samples at the Pb-L_{III}-edge (13055 eV). Four scans were taken per sample to increase the signal to noise ratio. The samples were cooled to liquid N_2 temperature to reduce the damping of the XAS signal due to thermal disorder. Data analysis was performed with the MacXAFS 4.0 package.

3. Results and Discussion

Figure 1 presents the Pb uptake on silica as a function of pH. A steep increase in Pb sorption is observed above pH=5.5. The first derivative spectra of the XANES data of the samples with pH>4.89 shown in Figure 1 are given in Figure 2, along with those of the reference compounds Pb^{2+} (aq) and $Pb_4(OH)_4^{4+}$ (aq). Comparison of the XANES spectra of the Pb/silica samples to those of the reference compounds indicates that at low pH, the local chemical environment of Pb adsorbed onto silica resembles that of Pb^{2+}(aq) whereas with increasing pH, the Pb/silica XANES spectra increasingly resemble that of $Pb_4(OH)_4^{4+}$ (aq). This indicates that at low pH, adsorbed Pb is

predominantly surrounded by water molecules. As pH increases, however, adsorbed Pb is increasingly coordinated to OH^- ligands. In terms of Pb adsorption mechanisms, this suggests that at low pH, Pb is mainly adsorbed in an outer-sphere fashion, whereas with increasing pH inner-sphere adsorption of Pb by coordination to surface OH^- ligands becomes increasingly important. Recent XAS studies by Strawn (1998) and Bargar *et al.* (1996), where Pb sorption was studied as a function of ionic strength and the type of surface sites respectively, have also shown the ability of Pb to form both inner-sphere and outer-sphere surface complexes at mineral interfaces.

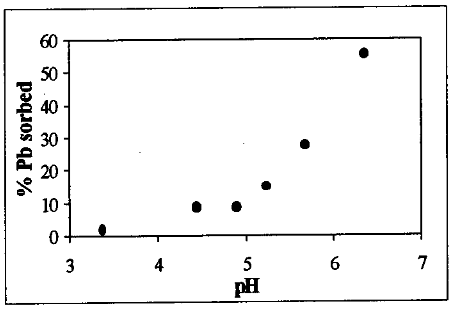

Figure 1. pH edge of Pb sorption on silica

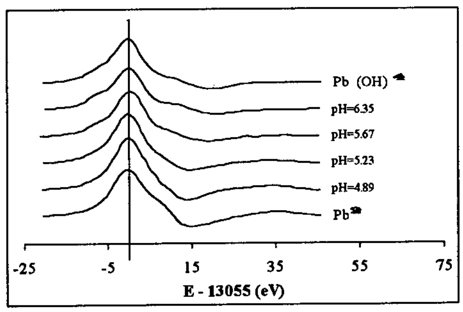

Figure 2. First derivative spectra of Pb/silica samples and Pb^{2+} and $Pb_4(OH)_4^{4+}$ reference compounds

4. Conclusions

XAS can be used to distinguish between adsorption mechanisms of Pb adsorbed on silica. At low pH values, Pb adsorption is mainly outer-sphere, whereas inner-adsorption becomes dominant at high pH. At intermediate pH values, both inner-sphere and outer-sphere Pb adsorption complexes occur at the silica surface.

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COMPETITIVE SORPTION OF CU AND PB TO HEMATITE: MACROSCOPIC, MODELING, AND EXAFS RESULTS

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1. Introduction

Iron oxides and oxyhydroxides are important sorbents for heavy metals in many aquatic and terrestrial environments. In polluted soils and sediments, several different metal contaminants typically occur together. Metal sorption to oxide surfaces has been the subject of numerous investigations, but information on sorption competition between different metal cations is scarce. The objectives of the present study were to (i) investigate competitive sorption effects between Cu^{2+} and Pb^{2+} on hematite (α-Fe_2O_3) particles, (ii) to test surface complexation models for their ability to predict competitive effects, and (iii) to verify the predicted speciation of sorbed metals at the molecular level using X-ray absorption fine structure spectroscopy (XAFS).

2. Materials and Methods

Hematite particles were prepared by aging a condensed Fe(III)-hydroxide gel for 72 h at 100 °C (Sugimoto and Sakata, 1992). The resulting particles were 122 ±29 nm in diameter and had a specific surface area of 28.3 m^2/g (N_2-BET method). The following approach was taken to investigate competitive sorption of Cu^{2+} and Pb^{2+}.

1.) The protonation behavior of the hematite surface was studied by acid-base titrations at different ionic strengths (0.005 to 0.5 M $NaNO_3$) using a computer-controlled titration set-up (Schudel et al., 1997). The titration data were modeled with a basic Stern model (BSM) and triple-layer model (TLM). Best-fit model parameters were obtained using FITEQL.
2.) Adsorption of Cu^{2+} and Pb^{2+} to the hematite was studied separately by batch experiments as a function of pH, ionic strength, and total metal concentration. Adsorption data at low to medium surface coverage (0.07 to 1.77 $\mu moles/m^2$) were used to calibrate surface complexation models for each metal separately.
3.) Competitive sorption of Cu^{2+} and Pb^{2+} was studied in batch experiments in which both metals were present at various concentrations. The results were compared with predictions based on the surface complexation models calibrated with titrations and single-metal data. Precipitation of oversaturated solid phases was included in the model calculations.
4.) Selected samples were analyzed by EXAFS spectroscopy in order to verify the speciation of sorbed metals at the molecular level. In this paper, we limit our discussion to the results for Cu. EXAFS experiments were conducted at beamline X-11A at the National Synchrotron Light Source, Upton, New York. Spectra were collected for moist pastes (room temperature) using a 13-element solid state detector with all channels tuned to the Cu-K edge. Data were analyzed using the MacEXAFS and FEFF software packages.

3. Results and Discussion

Acid-base titration and single-metal sorption data were modeled with basic Stern and triple-layer models, where the surface site density was systematically varied. Excellent fits were obtained with any site density between 2 and 20 sites per nm^2 (Christl et al., 1998). In contrast, predictions of competitive effects between Cu^{2+} and Pb^{2+} were very sensitive to the site density used in the model. Accurate predictions of metal sorption at higher surface coverage and competitive effects between Cu^{2+} and Pb^{2+} were obtained only if the site density was in the range between 6 and 10 sites per nm^2 and if precipitation of oversaturated solid phases was included in the model (Figure 1).

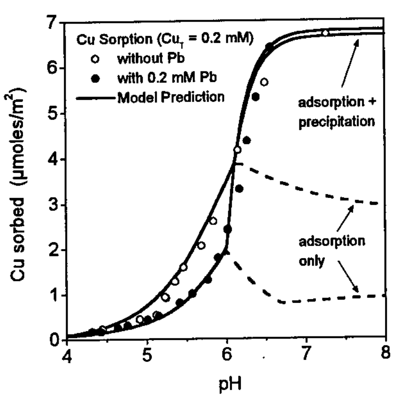

Figure 1. Sorption of Cu^{2+} to hematite in the absence and presence of Pb^{2+} (in 0.1 M $NaNO_3$). The solid concentration was 1 g/L. Lines represent BSM predictions based on titration and single-metal adsorption data, assuming a surface site density of 7.1 sites per nm^2.

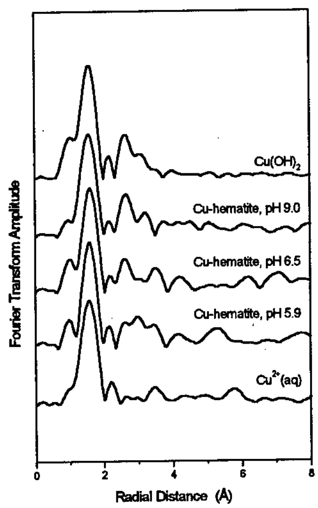

Figure 2. Radial structure functions resulting from Fourier transformation of EXAFS spectra for $Cu(OH)_2$, Cu adsorbed to hematite at three different pH values, and aqueous Cu^{2+} (16 mM $Cu(NO_3)_2$). Radial distances are not corrected for phase shift.

Figure 2 shows radial structure functions obtained from EXAFS analysis of Cu^{2+} sorbed to hematite at three different pH values. The concentrations of adsorbed Cu correspond to the data without Pb in Figure 1. Data for $Cu(OH)_2$ and aqueous Cu^{2+} are presented for comparison. EXAFS spectra and fitting results clearly indicated the presence of $Cu(OH)_2$ precipitation in Cu-hematite samples at pH 9.0. At lower pH values, EXAFS analysis suggested that inner-sphere complexation of Cu^{2+} at the hematite surface was the dominant mechanism, which is in agreement with our results from surface complexation modeling.

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KINETICS OF OXYANION SORPTION ON METAL OXIDES: A TIME-RESOLVED ATR-FTIR SPECTROSCOPIC STUDY

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1. Introduction

While sulfate and borate are important for both environmental and agronomic reasons, the chemistry of these oxyanions in soils is still poorly understood. The mechanism of sulfate sorption has often been the subject of debate, both historically and in the current scientific literature. A recent study (1) has determined that sulfate forms inner-sphere monodentate surface complexes with hematite at a fairly wide range of concentration and pH. While similar experiments in our research group have also seen inner-sphere complexes between sulfate and goethite at low pH, there is evidence of outer-sphere complexation at higher pH. At intermediate pH, an equilibrium between outer-sphere and inner-sphere complexes is seen. Ionic strength also plays an important role in this equilibrium, since far more outer-sphere adsorption occurs at lower ionic strength. A pH and ionic strength dependent mechanism of adsorption has also been observed with *o*-phthalate on metal oxides (2). While it is well accepted that borate forms inner-sphere complexes on metal oxides, the mechanism of boron adsorption is not completely understood. It is possible that there is a preference for either boric acid or borate, and that the rates of reaction for these species might be markedly different. The objective of our current research is to fully understand the effects that pH and ionic strength has upon the sorption behavior of borate and sulfate. To achieve this, we are currently utilizing ATR-FTIR spectroscopy to determine rates and mechanism(s) of complexation between oxyanions and metal oxides at conditions where one or more than one adsorption mechanism is occurring.

2.Materials and Methods

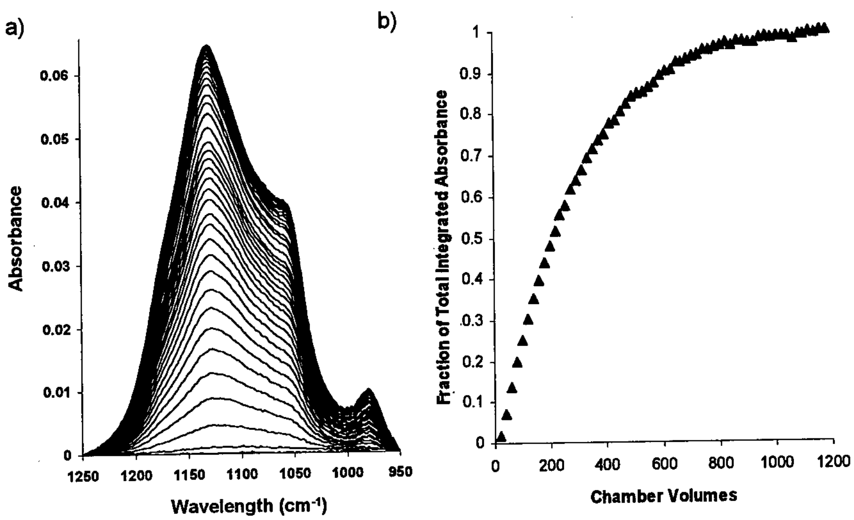
A Perkin-Elmer 1720X FTIR spectrometer equipped with a Whatman FTIR purge gas generator was used for all spectroscopic studies. Spectra were collected from a Spectra-Tech HATR accessory and flow cell, and a liquid N_2 cooled MCT detector was employed to achieve the sensitivity needed for these experiments. Approximately 2 mg goethite was deposited onto a 45° ZnSe ATR crystal. The dried deposit was rinsed with sodium chloride to remove poorly adhering goethite, and once dry was placed into the approximately 65 uL volume flow cell. Background electrolyte from a N_2 -purged pH-controlled reaction vessel was then pumped through the cell with a peristaltic pump until the system stabilized. A background spectra was then collected, reactant was added to the reaction vessel, and spectra were continuously collected until there was no noticeable change in the spectra.

3.Results and Discussion

Conducting experiments at extremes of pH and ionic strength allows one to establish reference spectra with only one surface complex present. Principal Component Analysis (a method of expressing the spectra of a complex mixture in terms of two or more standards) can then be used to

estimate the relative amount of each complex in samples of more intermediate pH and ionic strength where more than one complex is present.
We have learned via ATR-FTIR spectroscopy that the adsorption behavior of sulfate on goethite is unchanged over a wide range of initial concentrations for any given pH. This is in good agreement with the findings of Hug (1), and makes it possible to evaluate the time-dependence of sulfate reactions with goethite. By optimizing experimental parameters such as the number of co-added scans, resolution, mirror speed, and rate of flow through the flow cell, it is possible to follow the reaction of sulfate in real time (See Figure 1). This allows one to simultaneously determine both the rate parameters (by normalizing the integrated absorbance and fitting the data as a function of chamber volume) and the mechanism(s) of adsorption.

Figure 1 (a) Spectra of adsorbed sulfate as a function of chamber volumes through the flow cell. Reaction conditions were 0.01 N NaCl, pH 4.00, 25 μM SO_4^{2-} and 1.28 mL min^{-1} flow rate. (b) A plot of the integrated absorbance from 950-1250 cm^{-1} for the same experiment.



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COMPETITIVE RETENTION AND TRANSPORT OF VANADIUM IN SOILS

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1. Introduction

Vanadium is one of the metals where anthropogenic input has led to a significant enrichment of the element in the environment. Its mobilization as a consequence of man's industrial activities occurs mainly in the form of particles emitted into the atmosphere. Environmentally, the occurrence of vanadium in petroleum and coal is important because the combustion of these fuels constitutes the major source of vanadium emissions to the atmosphere (Lee, 1983). It has been shown that the burning of fossil fuels mobilizes 12000-24000 tones of vanadium per year (Bertine and Goldberg 1971). A large fraction of the vanadium-rich atmospheric particles may enter the soil environment as particulate fall-out or dissolved in rain. Vanadate and vanadyl are versatile at forming complexes that inhibit or stimulate activity of many enzymes by specific mechanisms. In general, vanadate behaves in a similar way as inorganic phosphate. Vanadium reduces acid phosphatase activity in soil, which alters the rate of mineralization of organic matter and may reduce phosphate bioavailabilty (Tyler, 1976). The interactions of V with nutrients in soils and its potential mobility in the soil profile have not been fully investigated (Mikkonen and Tummavuori 1994). The objective of the present study was to quantify the competitive kinetic retention behavior of vanadate and phosphate in two soils with contrasting properties and the interaction. Another objective is to assess the mobility of vanadium in soils where competitive retention is dominant. A third objective is to describe the competitive retention of P and V based on second-order two-site transport and retention models.

2. Experimental Methods

Soils: Two bench mark surface soils with contrasting properties were chosen for this study; a Sharkey clay (very fine, montmorillonitic, nonacid, Vertic Haplaquept) and Cecil loamy sand (clayey, kaolinitic, thermic Typic Hapludult). The Sharkey soil has 1.41 % organic matter content and pH of 5.9 whereas the Cecil soil has an organic matter content of 0.74% and a pH 5.6.

Transport: Miscible displacement experiments or column studies were carried out. Acrylic columns having a length of 10 cm and 6.4 cm ID were used. Sharkey or Cecil soil was packed into each column where a bulk density of 1.27 and 1.56 g cm^{-3}, respectively, were maintained. Each packed column was slowly saturated upward with 0.01N NH_4Cl background solution for 4d prior to V or P pulse applications. Each pulse contained 100 mg L^{-1} of V or P in 0.01N NH_4Cl background solution. An input flow velocity of 0.40cm h^{-1} using a piston pump was maintained. The effluent from each column was collected using a fraction collector. For each soil, several pulse applications of P, V, and/or P+V were made in a sequential manner. The characteristics of the different soil columns are given in Table 1. One column received a V pulse followed by a P pulse, then a pulse of a combined P and V solution. Another column received a P pulse followed by a V pulse, then a pulse of a combined P and V solutions. In a third column, two or three consecutive pulses of a combined P and V solution were made. Each pulse was approximately 7 pore volumes and was leached using 0.01N NH_4Cl background solution. To test whether equilibrium and/or kinetic V and P

retention are the dominant adsorption-desorption processes in soils, a 2 d period of no flow (or flow interruption) during pulse application and/or leaching were carried out.

Retention Kinetics: The traditional kinetic batch technique was used. Six initial (input) concentrations of V as NH_4VO_3 (5,10, 20, 40, 60, and 100 mg L^{-1}) in 0.01*N* NH_4Cl background solution were used to quantify the retention kinetics of adsorption for V by Sharkey and Cecil soils. In a separate experiment, P retention was also quantified for both soils and for a similar input concentration range. Adsorption was initiated by mixing 10 g soil with 30 g of the various V or P solutions in a 40-ml Teflon tube. Duplicates were used for each concentration. The slurries were shaken at 150 rpm and centrifuged at 4500 rpm for 10 min after specific reaction (sampling) times. A 1.0-ml aliquot was sampled from the supernatant at reaction times of 1, 2, 4, 8, 15, and 21d. The slurries were vortexed and returned to the shaker after each sampling. The collected samples were for analyzed for P and V using inductively coupled plasma (ICP) spectroscopy. To quantify competitive sorption of V and P, two sets of kinetic (batch) studies were investigated. In the first set, P was maintained constant at 100 mg L^{-1} and V concentrations were 5,10, 20, 40, 60, and 100 mg L^{-1}. In the second set, V was maintained constant at 100 mg L^{-1} and P concentrations ranged from 5 to 100 mg L^{-1} in a similar fashion. The times of reactions were also similar with 21 d adsorption maximum.

3. Results and Discussion

Sharkey soil exhibited very strong retention of V and fast reactions (or near equilibrium condition) appeared dominant. Although kinetic V sorption continued for 21 d, the amount of rapid V sorbed (with 24 h) accounted for some 90% of total sorption. For all input V concentrations, the presence of a constant P concentration (=100 mg L^{-1}) resulted in a slight but consistent decrease of the amount of V sorbed at all reaction times. Such a finding was consistent with that obtained for the kaolinitic Cecil soil, except that the extent of V retention was considerably less than that exhibited by the montmorillonitic Sharkey soil.

Miscible displacement results for Cecil soil showed that P was less retained by the Cecil soil compared to V. In addition, a P pulse was capable of displacing a significant portion of V already retained by the Cecil soil from an earlier pulse. However, the opposite was not as apparent when a P pulse followed by a V pulse. For both situations, the introduction of a pulse having P and V, BTC's for P arrived earlier and with higher peak concentrations than that for V. Due to flow interruptions, increases in the amounts of P or V sorbed were observed based on sharp decreases in concentrations. These observations are consistent for both P and V and illustrated the kinetic nature of the retention mechanisms. Efforts to describe the competitive retention of P and V based on second-order two-site models will be also be presented.

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A KINETIC APPROACH TO THE SPECIATION OF TRACE METALS IN SOILS

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1. Introduction

Regardless of their origin and the reasons for the increase in their concentration in soils, trace metals are liable to contaminate food chains by migrating towards ground water or by accumulating in plants. This possible mobility and bio-availability are the result of the reactivity of trace metals in soils, in other words their localization in different soil components that is now usually called speciation. Speciation of trace elements in soils may be performed using chemical methods which consists of using different chemical reagents for extraction of trace elements, terminating with their quantification in the extraction phase (normally when equilibrium is reached) [1]. Several reagents are generally used in what are called sequential extraction protocols [2]. However, everyone now agrees that these protocols cannot supply a reliable estimate of the speciation of trace elements in soils, particularly for thermodynamic reasons (measurements made at equilibrium)[2-4] so that, speciation performed when using a sequential extraction procedure is now only considered as operationnaly defined. This is why it appears necessary to consider other methods of determining this speciation of trace metals in soils in more detail, particularly considering kinetic aspects that also characterize the stability of the various trace metal - soil constituent associations [5]. Our aim was to study the feasibility of performing an operational kinetic speciation method applied to soil samples. This method should be able to provide, from the monitoring of the extraction of soil trace elements *vs* time with a given reagent (EDTA), the concentrations of the extracted trace metals from only two compartments, those quickly extracted (called labile) and those less quickly extracted (called not labile).

2. Materials and Methods

In order to have a large excess of EDTA (pseudo first order reaction), the initial concentration was 0.05 M. In most of the extractions, the acidity was near of neutrality (pH = 6.5) which permits to suppose that H^+ ions did not extract trace metals. From an experimental point of view, the extraction of trace metals was performed in a glass recipient with 20 g of sample and 1 l of the d'EDTA solution. These conditions correspond to a ratio m/v equal to 0.02 g/ml. Solution was stirred with a mechanical agitator with a speed of about 300 $tr.mn^{-1}$. The estimation of the different constants involved in the equation (1) was performed with a non linear regression program applied to the experimental curves (Marquardt algorithm).

3. Results and Discussion

If the reagent used for the trace metals extraction is in excess, we can consider that the reactions are pseudo first order reactions; the equation which corresponds to the evolution of the concentration of trace metals in the solution *vs* time will be thus given by :

$$Q(t) = Q_L\ (1-e^{-k_L t}) + Q_{NL}(1-e^{-k_{NL} t}) \qquad (1)$$

Where

- $Q(t)$ is the concentration of a given trace metal extracted at time t
- Q_L and Q_{NL} are respectively the amounts of trace metals initially bound to two sites (compartments) called labile and not labile
- k_L and k_{NL} are the kinetic constants associated two these compartements

One example of the results obtained for the proposed method is given on Table 1 and Figure 1. In order to assess the first elements of the interest of this speciation method, we applicated it to three series of different soil samples which were *a priori* different as to the trace metal speciation ; these samples came from one of the following groups : polluted soil samples, unpolluted soil samples and laboratory spiked soil samples.

1. Polluted soil samples. We can first observe that for these samples, EDTA behaves as a strong extraction reagent ; for most of the metals, it extracts more than 50 % of the total amount. Another observation for these samples and the studied cations is that the amount of labile cations is always significant : Q_L often represents more than 40 % of the total

amount and may reach 80 %. As a consequence, the not-labile fraction of the EDTA extracted metals is weak; it may be about 10 % of total cadmium for example.

2. Unpolluted soil samples. These soil samples reveal high amounts of trace metals; however, these levels are not due to human activities but have a geochemical origin. In this case, it is usually supposed that the total soil content of trace metals is not significantly related to the trace metal concentration in plants. The kinetic speciation method has been applied to some of these soil samples. Contrary to polluted soil samples, the labile fraction (Q_L) of trace metals provided by the speciation method is weak. Whatever the cation is, this fraction always less than 20 % of the total amount. Moreover, the not labile fraction (Q_{NL}) is more important that the labile fraction.

3. Laboratory spiked soil samples. In the last part of this study, the kinetic speciation method was applied to laboratory spiked soil samples. In this case, it is well known that trace elements which are added to soil samples in the laboratory, are generally mobile that is to say are not bound energetically to soil sites. If we consider the speciation of added cations (cadmium and copper), it was found for the studied samples that the fraction Q_L has increased (92 % of added cadmium, 90 % of added copper) while the fraction Q_{NL} has not changed. In other terms, added cations are, for the kinetic speciation method, considered as labile cations.

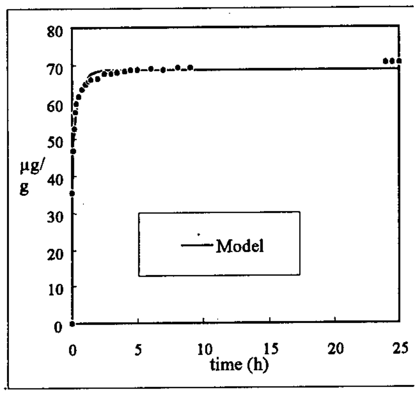

Figure 1: Kinetics of the extraction of cadmium from a polluted soil sample: comparison of experimental and calculated curves with Marquardt algorithm.

	Cadmium	
Compartment	Labile	Not-labile
Q (µg/g)	47	20
R^2	0.992	

Table 1 : Parameters of the kinetic model involving two compartments for the cadmium extraction with EDTA

4. Conclusions

The aim of this study was to assess the feasibility of soil trace metal speciation using a kinetic approach. It has been shown with different soil samples that, from a statistical point of view, the monitoring of EDTA extracted trace metals *vs* time may be used to differentiate fast extracted cations - called « labile » - and slowly extracted cations - called « not labile »; this approach requires some simple kinetic hypotheses and the calculation of four parameters using a non linear regression program. This speciation method does not involve classical soil compartments such as organic matter or iron oxides, for example, but is mainly an operational method. The application of this speciation method was performed to different soil samples for whith some hypotheses on the speciation of trace metals could be supposed *a priori*. For the speciation method, it was found that trace metals from soil with high levels of metals due to a geochemical origin are, as expected, not labile. On the contrary, for polluted soils or laboratory spiked soils an important part of the EDTA extracted trace metals was found to be labile.

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EXTENDED FREUNDLICH ISOTHERMS FOR METAL SORPTION IN SOILS: RELATIONS TO SOIL AND METAL PARAMETERS

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1. Introduction

From an ecological point of view the soluble fraction of nutrients and toxicants in soils is of main importance. The soluble part can be taken up by plants and can be leached into the groundwater. The buffer function of soils towards nutrients and toxicants can be characterized by the relation of the soluble fraction ("*I*ntensity") to a bigger pool of elements that is immobile ("*Q*uantity") either because of sorption on soil colloids or precipitation. Q/I relations in many cases can be described by the Freundlich isotherm $s = K\,c^{1/n}$, where s is the amount of solute sorbed by the soil sample in µmol kg^{-1}, c is the equilibrium solution concentration in µmol L^{-1}, and K and $1/n$ are constants. As soils are heterogeneous mixtures consisting of a broad variety of organic, organomineral, and mineral components as well as soluble substances, metal binding is highly variable. Thus, Q/I-relations are the complex result of the physico-chemical characteristics of the metals and the composition of the soils (BRUEMMER et al., 1986). With regard to soil parameters pH and organic matter content (C_{org}) are often characterized as the most important variables (HERMS & BRÜMMER, 1984). CHARDON (1984) and VAN DER ZEE and VAN RIEMSDIJK (1987), therefore, included pH and C_{org} into the Freundlich isotherm.

We conducted sorption and solubility experiments with nine metals (Mg, Cr(III), Co, Ni, Cu, Zn, Sr, Cd, Pb) and four soil samples of widely divergent composition. The main objectives were: (1) to relate sorption parameters of different metals to pH, organic C content *and* other soil characteristics; (2) to test whether the inclusion of physico-chemical properties of the metals is suitable in order to describe the sorption of different metals in various soils with one equation.

2. Materials and Methods

Batch experiments according to OECD-Guideline 106 (modified by KUKOWSKI, 1989) were carried out with four topsoil samples of different composition [pH ($CaCl_2$) 3.12-7.22; C_{org} 1.05-2.69%; clay 3.2-19.7%; Fe_d 1.34-8.34 g kg^{-1}]. The soil samples were spiked with 6 to 12 logarithmically increasing metal doses [in 0.01 M $Ca(NO_3)_2$; soil:solution ratio 1:5] and shaken for 40 h. Metal analyses in the filtrates were done with AAS, ICP-OES or ASV. The native metal proportion involved in sorption processes was characterized by an extraction with 0.025 M $(NH_4)_2$EDTA (pH 4.6). For further details see WELP and BRÜMMER (1998).

3. Results and Discussion

In a preceding paper (WELP and BRÜMMER, 1998) it was already outlined that the Q/I- relations of eight metals [except Cr(III) in two soils] are governed by sorption and complexation procecces and can be fitted by Freundlich isotherms. Sorption and solubility of the different metals are strongly influenced by the characteristics of the soil samples. Exept for Cr(III) the lowest concentrations in the liquid phase are observed in a Calcaric Regosol (pH 7.2). The adsorption capacity decreases in the order Eutric Cambisol (pH 6.0), Fimic Anthrosol (pH 4.8), and Haplic

Podzol (pH 3.1).The results confirm that pH is the main factor to control the partitioning of the metals between the solid and the liquid phase. A correlation analysis also indicates that further parameters [dissolved organic carbon (*DOC*), total organic carbon (C_{org}), Fe_d] influence the sorption of a metal in different soils. Therefore, a multiple regression analysis was performed in order to define the adsorbed metal amount (s) in our four soils as a function of the solution concentration (c) and various soil characteristics. The main variables of these "extended Freundlich isotherms" are c and pH. As a third parameter *DOC* is often more suitable than C_{org}. With the equation $\lg s = \lg K + a \lg c + b\, pH + c \lg DOC$ for seven metals very high values of determination are reached: Mg (r^2 = 0.966), Co (0.975), Ni (0.985), Zn (0.977), Sr (0.970), Cd (0.958), Pb (0.961). For Cu neither the inclusion of *DOC* nor C_{org} raises r^2 significantly. In the case of Cr(III) the influence of pH is neglectable.

Besides the influence of soil parameters sorption and solubility are also strongly metal specific. In our study the median Freundlich K values covered a range from 1.72 µmol kg^{-1} (Mg) up to 4250 µmol kg^{-1} (Pb) (WELP and BRÜMMER, 1998). FORBES et al. (1976) as well as HERMS and BRÜMMER (1984) pointed out that metal sorption increases with the ability of the metals to form hydroxo complexes. Thus, we tried to define the sorption of nine metals in four soils as a function of the solution concentrations, soil parameters *and* physico-chemical characteristics of the metals. We got an extended Freundlich equation which included the following terms: c, pH, pK_1 (equilibrium constants of the mono hydroxo species), IR (ionic radii of the metals), C_{org}; this way 81% of the variability of the sorbed amount (s) of all metals could be explained with a single extended adsorption isotherm.

4. Conclusions

Sorption and solubility of nine metals in soils are characterized by soil and metal specific peculiarities resulting in highly variable Q/I relations. The sorption of a single metal in different soils can be described with an extended Freundlich equation which includes soil pH and DOC or Corg. The sorption data of nine metals in four soils could be fitted with a corresponding equation which additionally included the equilibrium constants of the mono hydroxo species and the ionic radii of the metals.

More experiments are necessary to prove the general applicability of our findings. However, if concrete analytical results are missing, extended Freundlich equations seem to be an effective tool for estimating the sorption behavior on the basis of easily available physico-chemical data of the metals and general soil properties.

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MODELLING THE ACTIVITY OF METAL IONS (CD, ZN, CU) IN SOIL SOLUTIONS OF SOME CONTAMINATED SOILS: COMPARISON OF AN ADSORPTION AND A SPECIATION MODEL

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1. Introduction

An evaluation of the ecotoxicological risks of elevated metal contents in soils is difficult since those risks are related to the bioavailability of the metals, which in turn is influenced by their soil properties. The free metal ion activity in the soil solution appears to give a much better relationship with ecotoxicological effects. The aim of the study is to compare the results of two independently developed models with respect to the prediction of the metal ion activity in the soil solution.

2. Materials and Methods

The transfer function model (model I) is a Freundlich type adsorption model which uses soil solid phase characteristics together with pH and ionic strength of the soil solution. This model was developed within the framework of the ESQUAD study (Bril, 1995). The second model (model II) is a soil solution speciation model that emphasises the importance of complexation with dissolved organic matter (Römkens et al., 1996).

Soil solutions were collected by centrifugation from the topsoil (0-20 cm) of 20 sandy soils (soils from the waste water infiltration fields of the Zandleij near Tilburg, the Netherlands) which were contaminated by metal containing sewage effluents. Main soil parameters (% organic matter, % clay, CEC) were determined, together with total and 0.43 N HNO_3 –extractable metal content. The 0.43 N HNO_3 is used to give a reasonable estimate of total chemically active metal content. Soil solutions were analysed for total dissolved metal and soil solution parameters such as DOC, pH and macro-chemical composition. The data were used to calculate the metal activity in the soil solution with both models.

3. Results and Discussion

The soils varied in organic matter content from 2.7 % to 16 %, in CEC (cation exchange capacity) from 6 ceq.kg^{-1} up to 26 ceq.kg^{-1} . pH in the soil solutions was not very variable, rangeing from 5.2 to 6. Ranges of metal contents are given in the next table:

Metal	Solid Total (mg.kg^{-1})	Solid Active (0.43 N HNO_3)	Dissolved mg.m^{-3}
Copper	5.0 - 125	4.0 - 100	12 - 145
Cadmium	0.35 - 3.1	0.15 - 2.9	0.7 - 25.9
Zinc	40 - 550	36 - 545	135 – 2100

The results show good fits between both model calculations for all three metals. As an example, figure 1 shows the calculated activity of zinc plotted against the distance of the sample location aafrom the effluent source. Since the models essentially give the same results, we conclude that both models can be used to estimate soil solution metal activity for these soil samples.

4. Conclusions

In general, we expect that metal ion activity can be estimated with a transfer function (model I) without elaborate soil solution generation when pH and ionic strength of the soil solution can be estimated, nd the main soil solid composition is known. This way existing databases of soil properties can be used to assess the bio-availability of the heavy metals. An example of this approach is presented in the accompanying article (Groenenberg et al., 1999)

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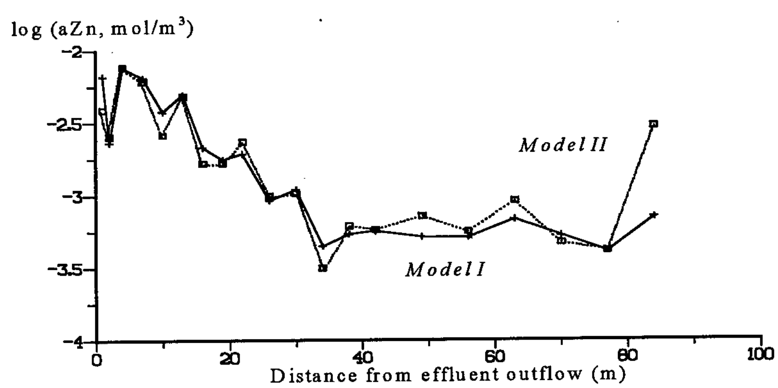

Figure 1: Calculated zinc activity (+ = model I, □ = model II)

ADSORPTION/DESORPTION HYSTERESIS OF TRACE METALS WITH SOIL COMPONENTS: A DYNAMICAL SYSTEMS APPROACH

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1. Introduction

Fundamental mistakes may arise in quantitative descriptions of adsorption/desorption processes in soils through a failure to recognize both the equilibrium and kinetic aspects of hysteresis. The object of our study was to model the interaction of copper with soil components utilizing a dynamical systems approach to incorporate these features. Such dynamical systems approaches have proven fruitful in revealing essential features of sorption phenomena (Oppenheimer, 1995).

2. Materials and Methods

Equilibrium hysteresis model: We consider a system displaying hysteretic adsorption/desorption under equilibrium conditions, where the adsorption and desorption isotherms are given by $q = f_s(c)$ and $q = f_d(c)$. This system with: 1). an initial state of (c_o, q_o) in a region outside the adsorption isotherm will approach an equilibrium state of (q_f, c_f) satisfying $q_f = f_s(c_f)$; 2). an initial state of (q_f, c_f) in a region outside the desorption isotherm will reach an equilibrium state of (q_f, c_f) satisfying $q_f = f_d(c_f)$; and, 3). a state of (q_f, c_f) within a region delineated by the adsorption and desorption isotherms is at equilibrium. We assume both adsorption and desorption equilibrium isotherms are of the general Langmuir-Freundlich type,

$$f_s = \frac{\alpha_s c^{\gamma_s}}{1+\beta_s c^{\gamma_s}} \quad \text{and} \quad f_d = \frac{\alpha_d c^{\gamma_d}}{1+\beta_d c^{\gamma_d}},$$

where numerical identification of unknown parameters α_s, β_s, γ_s, α_d, β_d, and γ_d is done using experimentally determined adsorption and desorption isotherms. If there is a refractory sorbed concentration, q_r, a modified Langmuir-Freundlich equation, with unknown parameters α_d, β_d, and γ_d, is used to numerically fit experimental desorption data.

Kinetic hysteresis model: For a system not at equilibrium with time dependent solution concentration, $c(t)$, and sorbed concentration, $q(t)$, the rates of change of these concentrations are assumed to satisfy the system of differential equations,

$$\begin{cases} \frac{dc}{dt} = -r_{cs}\left[q(t) - f_s(c(t))\right]^- + r_{cd}\left[q(t) - f_d(c(t))\right]^+ \\ \frac{dq}{dt} = +r_{qs}\left[q(t) - f_s(c(t))\right]^- - r_{qd}\left[q(t) - f_d(c(t))\right]^+, \end{cases}$$

where r_{cs}, r_{cd}, r_{qs}, r_{qd} are rate constants and $[x]^+ = \max(0, x)$ and $[x]^- = \max(0, -x)$ for any real number x. Our analysis permits observation of hysteresis even in cases where $f_s = f_d$, since there may be differences in the rates of adsorption and desorption, $r_{-s} \neq r_{-d}$, or purely kinetic hysteresis. The region bounded by the adsorption and desorption isotherms along with the isotherms themselves

is the set of equilibrium points for the dynamical system defined by the differential equations above. Data plotted in the *c&q* phase plane along with isotherms illustrates that as time increases, adsorption approaches equilibrium along a straight line in the region outside the adsorption isotherm, similarly desorption approaches equilibrium through the region outside the desorption isotherm. This phase-plane analysis readily permits determination of equilibrium conditions.

Experimental: Two smectite clays, Wyoming and Mississippi, were pre-treated for removal of soluble salts, carbonate, organic matter and iron oxides with the usual procedures. The <2-Φm fraction was saturated with Mg salt. Adsorption isotherm experiments were conducted by reacting 50-mg sub-samples of clay with $Cu(NO_3)_2$ solutions from 0.2 to 300 mg Cu L^{-1}. After two days, supernatant Cu concentrations were measured. Kinetic experiments were performed similarly by reacting clay with either 5, 20 or 100 mg Cu L^{-1} while maintaining constant ionic strength. Supernatant Cu was measured after 3, 8 , 15, and 30 min, and 1, 2, 4, 14, 24, and 48 hr. Equilibrium and kinetic desorption data were obtained by reacting clays, previously equilibrated with various amounts of Cu, with Cu-free solutions and measuring supernatant Cu.

3. Results and Discussion

Data from equilibrium adsorption experiments with Wyoming smectite, conformed to a Freundlich-form of f_s with $\alpha = 3.999$, $\beta = 0$, and $\gamma = 0.386$. Phase-plane analysis, as represented in the figure, using an initial concentration of 5 mg L^{-1}, revealed that equilibrium had not been attained, even with a reaction time of 48 hours. Calculated adsorption rate constants were 6.6×10^{-3} min^{-1} for r_{qs} and 1.93×10^{-5} Φg Cu per mg clay min^{-1} for r_{cs}. Oftentimes least-squares fitting methods will identify local minimum generating parameters. The use of a quadrature initialization method, developed by the authors for rate coefficient identification (Oppenheimer et al., 1998), appeared to sucssefully prevent such false minima . Moreover, our experimentally derived data sets from adsorption and desorption provides the basis for formulation of a kinetic multi-component approach (Selim and Amacher, 1997). This multi-component approach is based on the kinetic hysteresis model after incorporation of the general Langmuir-Freundlich equations presented above. Evaluation of this approach and a sensitivity analysis is also discussed.

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SIMULATED IN-SITU CHEMICAL IMMOBILIZATION OF HEAVY METALS IN CONTAMINATED SOILS

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1. Introduction

Metal mining and smelting operations in Northeastern Oklahoma have produced extensive tracts of contaminated soil with high concentrations of cadmium (Cd), lead (Pb), and zinc (Zn). Leaching of heavy metals from smelter contaminated soils and wastes has caused surface and groundwater contamination at many smelting and mining sites. Chemical immobilization treatments that adsorb or precipitate Cd, Pb, and Zn in soil solution could prevent transport of heavy metals from contaminated soils, and serve as an active barrier to prevent metal seepage from smelter sites. The objective of this study was to evaluate and compare the ability of chemical immobilization treatments to reduce heavy metal mobility in smelter contaminated soil. Three chemical treatments were evaluated for reducing the mobility of Cd, Pb, and Zn: (AL) agricultural limestone [$CaCO_3$], (RP) rock phosphate [$Ca_5(PO_4)_3OH$], and (DAP) diammonium phosphate [$(NH_4)_2HPO_4$].

2. Materials and Methods

Soils with elevated residual concentrations of cadmium, lead, and zinc were collected from a defunct smelter site in Northeastern Oklahoma, USA. The total level of metal contamination in the soil was determined by X-ray fluorescence (XRF) with contents of 152 mg As kg^{-1}, 1090 mg Cd kg^{-1}, 2100 mg Cu kg^{-1}, 5150 mg Pb kg^{-1}, and 69200 mg Zn kg^{-1}. The soil was also extracted using the Toxicity Characteristic Leaching Procedure (TCLP, SW-846 Method 1311) (USEPA, 1986) to determine its hazardous waste disposal status. TCLP extractable Cd (39.94±1.59 mg L^{-1}) and Pb (21.02±0.58 mg L^{-1}) from the study soil exceed regulatory levels for Cd (1.0 mg L^{-1}), and Pb (5.0 mg L^{-1}) (mean ± 1 SD; n=4).

Chemical immobilization treatments were evaluated using miscible displacement experiments with repacked soil columns (Selim et al., 1990). Treatments were: (1) Agricultural Limestone (AL) – 170 g kg^{-1} mixed with soil; (2) Rock Phosphate (RP) – 180 g kg^{-1} layered on top of soil, 60 g kg^{-1} and 180 g kg^{-1} mixed with soil; (3) Diammonium Phosphate (DAP) – 2 g kg^{-1}, 4 g kg^{-1}, 10 g kg^{-1}, and 90 g kg^{-1} mixed with soil. Soil solution fractions were collected through 60 to 70 pore volumes and analyzed for pH, metals by ICP-AES, and anions by ion chromatography. Conservative trace analysis (Br) was performed following each experiment. Metal breakthrough curves were plotted for comparison between treatments and calculation of total mass of metals eluted through 60 pore volumes.

3. Results and Discussion

Bromide breakthroughs curves showed behavior representative of a conservative tracer in porous media and are well described using the classical convection-dispersion equation. DAP treatments were the most effective for immobilizing Cd, Pb, and Zn eluted from the contaminated soil when

compared with the untreated check (Table 1). DAP treatments reduced the total metal eluted up to 95% for Cd, 99% for Pb, and 96% for Zn. DAP treatments mobilized As from the contaminated soil by phosphate-arsenate ligand exchange. Increasing the DAP application from 2 to 10 g kg^{-1} reduced Cd and Pb eluted but also increased As eluted from 3 to 130 µg kg^{-1} over 60 pore volumes. RP layered at 180 g kg^{-1} showed a 99% reduction in Pb eluted compared with the untreated check, but was less effective for Cd and Zn (<25% reduction). RP mixed treatments proved to be ineffective for reducing Cd, Pb, and Zn leached from the contaminated soil. Chemical immobilization with phosphate materials is consistent with the formation of insoluble lead phosphates, such as pyromorphite and chloropyromorphite. Overall, more soluble DAP provided better reduction in heavy metal mobility than less soluble RP. Cooper et al. (1998) and Hettiarachchi et al. (1997) also reported soluble phosphates were more effective for immobilizing heavy metals. Compared with DAP, AL treatment was not as effective for reducing the cumulative mass of metal eluted form the soil. The AL treatment increased soil pH from 6.8 to 7.2. This small increase in pH had little effect on metal solubility.

4. Conclusions

Soluble phosphate as diammonium phosphate provided the best overall immobilization of Cd, Pb, and Zn. Optimal DAP application should be based on reducing soluble Cd, Pb, and Zn, and minimizing the solubility of As. Metal phosphate precipitates with low solubility products indicate long-term perseverance in the soil environment. Probable metal phosphate precipitates predicted by chemical speciation models will be presented.

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Table 1. Cumulative mass of As, Cd, Pb, and Zn (per kg of soil) collected from miscible displacement experiments through 60 pore volumes of elution.

Treatment	Trtmt. Rate (g kg^{-1} soil)	As (mg kg^{-1} soil)	Cd (mg kg^{-1} soil)	Pb (µg kg^{-1} soil)	Zn (mg kg^{-1} soil)
None	0	0	14.9	460.3	107.6
DAP	2	0.003	7.1	236.5	5.7
DAP	10	0.13	0.8	5.2	4.5
RP Mixed	60	0	12.7	418.0	94.1
RP Mixed	180	0	11.8	406.6	85.5
RP Layer	180	0	7.0	0.5	61.6
AL Mixed	170	0	6.7	252.2	53.7

LABILITY OF HEAVY METALS IN SOILS: ROLE OF SOIL PROPERTIES

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1.Introduction

Establishment of soil screening levels for risk assessment and protection of groundwater relies on an understanding of the lability of chemicals in soils. It has been well documented that the lability (mobility and bioavailability) of heavy metals varies significantly with soil properties for a similar total concentration. Thus, identification of the major soil parameters affecting metal labilities in soils is requisite to predication of metal behavior and establishment of appropriate soil screening levels. This study investigated desorption of three metals, Cu, Ni and Zn, from fifteen soils. The major soil parameters determining the desorption of these metals from soils were elucidated. Models were developed to predict the partitioning of metals to soil solution and aqueous speciation.

2.Materials and Methods.

Fifteen New Jersey soils with texture ranging from sand to loam and organic C content from 1.2 to 49.9 g/kg were employed to conduct the experiments. The soil samples were air-dried and sieved through a 2-mm screen before use. Detailed characteristics of the soils were reported by Yin et al. (1996). The total concentrations of metals in soils were determined by acid digestion following the U.S. EPA SW-846 method (1996). Desorption of metals from soils was initialized by mixing each soil with deionized water at natural soil pH without lab amendment of soils with metals of interest. The soil mixtures were equilibrated by shaking on a reciprocal shaker at 100 strokes per minute for 24 hours at 25 ± 1□C. After equilibration, soil solids were separated from solution by centrifugation followed by filtration through a 0.45 μm pore size membrane filter. The final pH for each filtrate was determined by an Orion pH electrode. The concentrations of soluble metals and dissolved organic C in the filtrates were determined by a Spectro ICP and a Dohrman DC-90 TOC analyzer, respectively. The free Cu^{2+} activities in the filtrates were determined by an Cu ion selective electrode.

3.Results and Discussion.

A preliminary study indicated that increases in the water:soil ratio decreased the partition coefficient K_d (calculated as the ratio of metal adsorbed per mass of soil/ soluble metal concentration). To mimic field conditions, we focused on studies at a minimum water/soil ratio of 20 mL/25 g, which was close to field capacity and was also lab operable. The results indicate that soil pH and organic matter are the major parameters determining the partitioning of these metals to soil solution and aqueous speciation. Among the three metals studied, Cu has strong binding affinity for both soluble and particulate organic matter. Consequently, soil-water partitioning of Cu largely depended on the partitioning of organic matter between soil solids and solution phases. The partition coefficient for soluble Cu correlated well with the partition coefficient for organic matter with a regression coefficient R^2 of 0.91 (Figure 1). The partitioning of free Cu^{2+} ion, however, was highly pH dependent and also closely related to the total soil organic C content. Normalization of the partition

coefficient for free Cu^{2+} (calculated as the ratio of Cu adsorbed per mass of soil to the concentration of free Cu^{2+} ion) with the soil organic C content significantly improved the correlation between the partition coefficient for free Cu^{2+} ion and pH with regression coefficient R^2 increasing from 0.80 to 0.96 (Figure 2).

Unlike Cu, the effect of dissolved organic matter on the partitioning of Ni and Zn was not important compared with that of particulate organic matter at the natural soil pH. The partitioning coefficient increased with soil pH for both metals. Yet, the partitioning of both metals was affected by soil organic C content. Normalization of the partitioning coefficient with soil organic C content significantly improved correlations between the normalized partition coefficient and pH for both metals with regression coefficient increasing from 0.53 to 0.87 for Ni and from 0.62 to 0.83 for Zn.

4.Conclusions.

This study demonstrated that soil pH and organic matter are the two most important parameters determining metal partitioning and aqueous speciation in soils. By incorporating both soluble and particulate organic matter and considering the effect of pH independently, correlations can be developed to predict metal partitioning and speciation in soils.

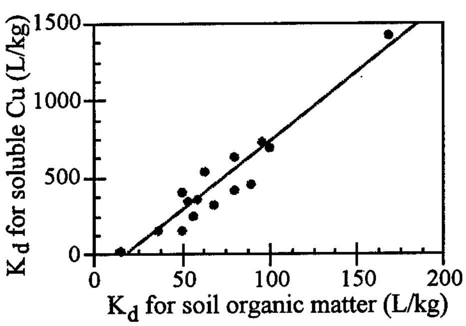

Fig. 1 Partition coefficient for soluble Cu as a function of the partition coefficient of soil organic matter for fifteen New Jersey soils. Soils were at their natural pH and no Cu was added. Soil:water = 25 g/20 mL; T = 25□C. Regression coefficient R^2 = 0.91.

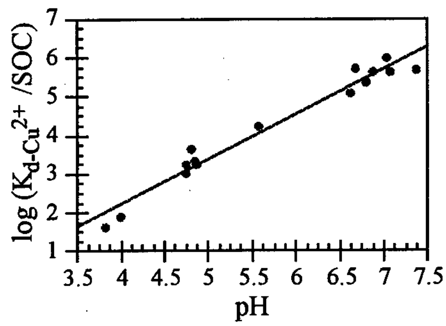

Fig. 2 Organic matter normalized partition coefficient for free Cu^{2+} ion as a function of pH for desorption of copper from fifteen New Jersey soils. Soils were at their natural pH and no copper was added. Soil:water = 25 g/20 mL; T = 25□C. Regression coefficient R^2 = 0.96.

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SORPTION-DESORPTION PROCESSES OF SELENIUM IN SOILS OF MEDITERRANEAN AREAS

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1. Introduction

The environmental hazards caused by heavy metals are closely related to their mobility and concentration in the soil solution. An increase of their concentration in the liquid phase induces an higher uptake by plants and increases leaching, while the pollution risk is strictly reduced if the metals are adsorbed by solid phase (PETRUZZELLI, 1997). These remarks are valid also for selenium, which has physical and chemical properties intermediate between those of metals and non-metals and which plays an important role both in animal and plant nutrition. The physical, chemical and biological characteristics of soil affect the distribution of the element between the solid and liquid phases in soil determining the mobility and availability of different Se forms (SINGH et al., 1981). Leaching of the soil profile could result in the mobilization of significant quantities of Se which in turn may achieve hazardous concentrations in surface, drainage, and ground water (NEAL et al., 1989). The present work was undertaken to study the adsorption/desorption processes of selenate on different soils, typical of the Mediterranean areas where the biogeochemical processes of Se are not well known.

2. Materials and Methods

The experiments were carried out using four soils characterized by different physical and chemical properties (Table 1). All samples were collected from the surface to 15 cm deep, air-dried and sieved to obtain the fraction <1 mm. To determine adsorption isotherms of selenate, 2.5 g of soil were shaken for 12 hours with 25 ml of solutions containing 1, 3, 5, 10, 20, 40, 60, 80 or 100 ppm of Se as Na_2SeO_4. The pH of the solutions varied from 5.3 to 5.5. At the end of the shaking period the pH was measured, suspensions were centrifuged, and the supernatants were filtered. The samples were then acidified with concentrated HCl for analysis of Se. Desorption isotherms were determined resuspending the samples in phosphate solution (0.1 M KH_2PO_4 in distilled water, pH 5.0) under the same experimental conditions as for selenate adsorption. The suspensions were then filtered and the filtrates were analyzed for Se by a Varian atomic absorption spectrophotometer equipped with a VGA 77, vapour generation accessory.

Table 1: Some characteristics of the soils used

		arable	cultivated	woodland	pine forest
clay	(%)	7.65	21.0	7.10	12.5
organic matter	(%)	0.52	1.59	5.83	10.7
pH (H_2O)		8.0	7.5	4.5	7.4
C.E.C.	(meq/100g)	11.25	14.37	30.6	27.5

3. Results and Discussion

The sorption data were analyzed by the Freundlich equation:

$$q = KC^{1/n}$$

where **q** is the amount of selenium adsorbed on unit weight of soil ($\mu g\ g^{-1}$), **C** the equilibrium selenium concentration ($\mu g\ ml^{-1}$), and **K** and **1/n** are empirical parameters related to adsorption capacity and intensity, respectively. These constants characterize the system, but are not based on assumption of a specific sorption

mechanism. The Freundlich equation, which parameters are reported in Table 2, does not allow to calculate the adsorption maxima, but differences in the sorption capacity of different soils can be estimated by the comparison of the K values.

Table 2: Freundlich parameters for selenate adsorption.

type of soil	K	n	r
arable	3.74	1.35	0.978
cultivated	6.10	1.40	0.981
woodland	9.78	1.51	0.996
pine forest	15.96	1.84	0.979

The ability in adsorbing selenate and the isotherm patterns varied considerably in the four soils. In pine forest and woodland soil, the isotherms can be classified as 'L type': the amount of selenium sorbed appeared to move towards saturation, indicating that all possible available sites have been filled. These soils, which were characterised by the highest organic matter content, showed a great affinity for the element, which is reflected in the higher K values. The highest $CaCO_3$ content of the pine forest soil (12.9%) contributed in increasing the Se adsorption even at high pH value. The pattern of the sorption isotherms in the other two soils was quite different. The sorption can be described by an 'S type' curve. At low concentrations the affinity of the solid phase was less than that of the liquid phase. Increasing the concentration of selenium in solution, the affinity of the solid phase increased and the sorption was favoured. The reduced sorption capacity is reflected by the lower values of the K parameter of the Freundlich equation. Results indicated that the organic matter content played the most important part in the adsorption of selenium, while pH appeared to not greatly affect the soil's ability to adsorb Se. However, the arable soil showed the lowest sorption, probably due to its high pH, which kept high the solubility of selenium. Results concerning the Se desorption indicated that process is not complete. The desorption isotherms are not the same as the adsorption and these differences might suggest the existence of hysteresis or irreversible adsorption. This phenomenon could be a result of a permanent change in the soil-selenate system, for instance a structural rearrangement of adsorbing surfaces which were different from those during adsorption.

4. Conclusions

Results obtained from our sorption experiments showed the relevant sorptive capacities of the investigated soils, particularly in relation to the organic matter content. Whether sorbed selenium can be desorbed and become available for environmental processes, is a key problem to evaluate. Desorption experiments indicated that phosphate desorbs Se into the solution phase, but a noticeable portion of the sorbed selenium is irreversibly retained. This suggests the existence of linkages which allow the release of selenium in the soil solution only after physico-chemical variation, such as exchange with phosphate ions. The fraction resistant to extraction by phosphate solution represent a substantial reservoir of Se in the soil that may be resistant to leaching.

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MOBILITY AND SPECIATION OF ARSENIC IN CONTAMINATED SOILS FROM TWO DIFFERENT INDUSTRIAL SITES

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1. Introduction

Because arsenic solubility, mobility, bioavailability and toxicity depend on its oxidation states (Masscheleyn et al., 1991), studies of As speciation and mobility are essential to understand the As behaviour. The main forms of As in soils are arsenite (As(III)) and arsenate (As(V)). As(III) is the most toxic species found in soil and is more mobile than the As(V).
Adsorption experiments show that As sorption is dependent on pH, Eh and that the metal is linked to the nature of soil components (oxyhydroxides, clays, calcite). Moreover the concentration of some anions (PO_4^{3-}, for instance), which can compete with the different adsorption sites, is an important factor (Livesey and Huang, 1981 ; Pierce and Moore, 1982 ; Manning and Goldberg, 1997).
The aim of the work is to understand the As behaviour in some polluted industrial soils. It is composed of three parts : (i) the soil characterization, (ii) the identification of bearing phases and the chemical As speciation (iii) the study of As mobility and As desorption with batch experiments.

2. Materials and Methods

The three soils (Sa, Sb and Sc) used in this study were collected from two different industrial sites heavily polluted by arsenic due to anthropogenic sources (production of pesticides and herbicides for Sa ; gold mine for Sb and Sc). Soil samples were collected in the surface soil (0 to 40 cm) and were homogenized, air-dried, 2 mm sieved and stored at room temperature in plastic containers. The experimental procedure used for the soil characterization is explain in the figure 1.

I / Soil preparation

- Soil homogenization of each 200 kg soil (quartering)
- Air drying
- Sieving at 2000 µm

II / Soil sample characterization

- Physical and chemical analysis
- Fraction study and separation :
  2000 µm / 500 µm - 500 µm / 200 µm - 200 µm / 50 µm - 50 µm / 20 µm - < 2µm →

III / Fraction characterization

- Mineralogical analysis
- Physical and chemical analysis
- Chemical speciation

Figure 1 : Scheme of the experimental procedure for the soil characterization

Total As was determined by ICP-AES. Extraction for speciation studies were done using 1 M phosphoric acid and microwave digestor, and the analysis of the acidic extracts done by HPLC-ICP-MS (Thomas et al., 1997).
Batch experiments were carried out and the main parameters which have an influence on the arsenic behaviour in soils were studied : pH (3, 5, 7, 9, 11) ; [PO_4^{3-}] (10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M).

3. Results and Discussion

Tables 1 and 2 and figure 2 gather the main properties of the soils.

	pH	pH KCl	CEC (*)	TOC(♦)	As (ppm)
Sa	6.78	6.14	10.7	8.43	13500
Sb	7.26	6.54	11.5	5.54	9400
Sc	3.83	3.16	15.4	0.34	11130

(*) meq/100g soil
(♦) Total Organic Carbon (%)

Table 1. Soil properties

	Sa (%)	Sb (%)	Sc (%)
2000 / 500 μm	28.9	27.1	46.6
500 / 200 μm	26.1	30.9	19.9
200 / 50 μm	23	24	9.5
50 / 2 μm	18.2	15.4	18.9
< 2 μm	3.8	2.6	5.1

Table 2. Fraction size classification (weight %)

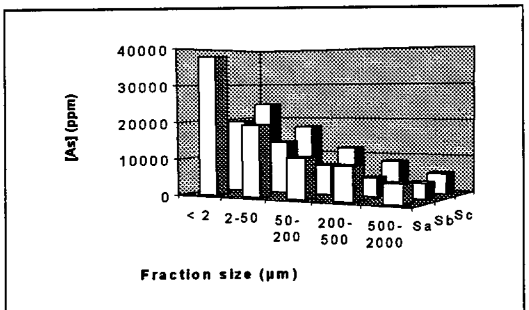

Figure 2 : As concentration in different fractions

These soils are sandy without any other metal pollution than As.
As is preferentially observed in the finest fractions size.
Concerning As forms, we put forward that both As(V) and As(III) are present in two of the three soils. Nevertheless As(V) represents more than 90% of the total As (Sa : 96.94%, Sb : 91.81%, Sc : 100%).

As(III), present in Sa and Sb, is not detected in the < 2 μm fraction size and don't have the same distribution than As(V) which is present in the finest fraction size. Furthemore, the As(III) distribution is different in Sa and Sb samples.
Some leaching tests with water have allowed to extract 4% of total As. Other batch experiments are in progress in order to study the influence of pH and PO_4^{3-} contents on the mobility and speciation of arsenic.

4. Conclusions

The soils studied have different physical and chemical properties and arsenic pollution nature.
As(V) and As(III) are not distributed with the same way among the different fractions. These differences in As compounds should let us supposed that the mobility of the polluant won't be the same for the two differents soils. This specific As behaviour has been put in light even for the two different samples of the same site.

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Metal Organic Interactions
(Special Symposium 10)

PATHWAYS OF METAL MOBILIZATION BY HUMIC AND FULVIC ACIDS STUDIED USING NATURAL ORGANIC MATTER

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1. Introduction

Pathways of metal mobilization by fulvic and humic acids were investigated using amorphous oxides of Fe and Al. These experiments estimate the relative contributions to metal solubility by the formation metal-organic complexes, stabilization of colloids, and reduction—in the case of iron. A schematic model of the interactions and their relative importance is presented.

2. Materials and Methods

Mobilization of Fe or Al amorphous hydrous oxides (AHO) by natural organics was investigated in batch experiments under sterile conditions. Humic and fulvic acids were collected from a diversity of sources following the XAD-8 resin method (Leenheer 1981). The experiments were initiated by mixing a suspension of laboratory-synthesized Fe- or Al-AHO with an equal volume of solution containing fulvic or humic acid, and maintained at 25 C in a glass-jacketed beaker. The pH was maintained with a pH-stat setup and the suspension was mixed continuously using Teflon stir-bars and a magnetic stirrer. Subsamples taken over time were fractionated using Nuclepore polycarbonate membrane filters with 0.4, 0.1, or 0.05-μm pores. Dissolved organic carbon (DOC) and metal concentration were measured in the fractions using a low-temperature oxidization and ICP, respectively. Reduced Fe was measured using a procedure optimized to avoid interference from colloidal iron.

3. Results and Discussion

Primary pathways for metal mobilization are shown for Fe in Figure 1. The effects of fulvic and humic acids were similar for the Al and Fe AHO experiments when reduction reactions were not considered. After mixing the fulvic/humic acid and AHO, adsorption occurred rapidly via ligand exchange reactions, thereby releasing hydroxyl ions. Most DOC adsorption occurred within minutes after mixing but DOC continued to decrease over longer periods until a minimum was reached within a few days. DOC removal was greater for humic acids. After the initial drop in DOC, it remained constant in the Al experiments, while the DOC increased slowly after the minimum was reached in the Fe experiments.

The adsorbed organics may follow one of several pathways. It may simply remain as an adsorbed species with litter further reaction. The fulvic/humic acids may form multiple bonds thereby bridging particle and preventing the floc from dispersing. Over time, Fe with adsorbed organics may breakdown via reductive dissolution and form Fe-II and smaller metal-organic colloids.

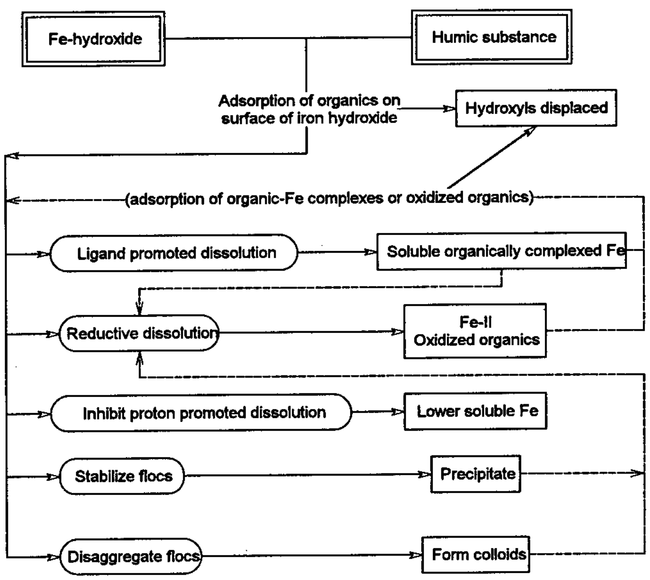

Figure 1. Interaction pathways documented for Fe-hydroxide and their mobilization/immobilization products.

4. Conclusions

Long term release of adsorbed DOC occurred in the Fe-AHO experiments due to reductive dissolution of Fe coupled with oxidation of the adsorbed organics. For the fulvic and humic acids, the formation of Fe-II was ultimately the dominant soluble form of Fe. The Al-AHO experiment indicated less colloid formation and greater DOC removal, possibly due to the greater solubility of Al; as more Al is dissolved, it may cause the organics to be precipitated. The ability of the organic acids to mobilize AHO was source dependent while fulvic and humic acid isolated from the same source displayed similar ability to mobilize Fe-hydroxide, although substantially more humic acid was adsorbed.

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OXIDATIVE DIAGENESIS OF METAL BINDING STRUCTURES IN NATURAL ORGANIC MATTER

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1. Introduction

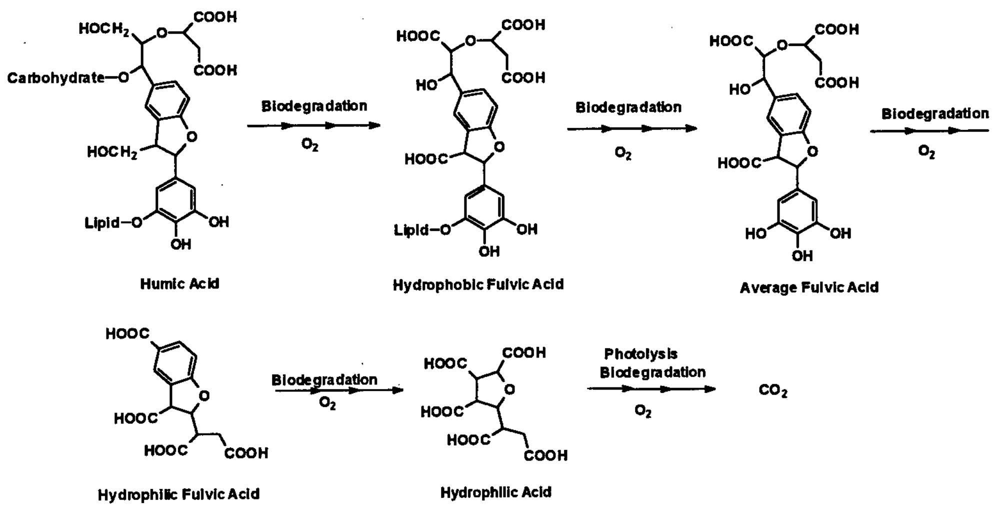
Natural organic matter contains oxygen, nitrogen, and sulfur functional groups that bind a variety of metals in soils, sediment, and water. Many metal-binding structures in precursor plant biomolecules such as enol, flavonoid, orthohydroxy quinone, phenol, tetrapyrrole, and thiol structures act as antioxidants (Larson, 1997), and lose much of their metal-binding characteristics when they are oxidized. Other metal-binding groups such as the primary amino group in amino acids, peptides, and proteins, react with sugars to form complex humic substances in the Maillard condensation reaction and again, the metal binding properties are altered by the reaction. The objective of this report is to combine studies of humic substance structures, studies of humic-metal binding characteristics, and studies of biotic and abiotic oxidative diagenesis of natural organic matter to determine how metal-binding structures form in oxic environments. The focus will be on oxygen functional groups because nitrogen and sulfur functional groups are largely deactivated by oxidation and condensation reactions in oxic aquatic environments (Larson, 1997).

2. Materials and Methods

A number of structural studies were conducted on dissolved organic matter isolated from tropical, temperate, and arctic environments in waters of varying trophic status, with organic matter inputs that varied from allochthonous to autochthonous. (Leenheer, 1994). Average structural models were derived for these organic isolates that incorporated elemental analyses, number-average molecular weights, and quantitative ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR, and infrared spectrometric data.(Leenheer, 1994; Leenheer et al, 1994; Leenheer et al, 1998). A fulvic acid, isolated from the Suwannee River, Georgia, was intensively studied and average structural models were derived to describe its metal-binding characteristics (Leenheer et al., 1998).

3. Results and Discussion

Substituted succinic acid structures were found to be important for describing metal binding properties (Leenheer et al, 1998) of fulvic acid from the Suwannee river. Structural models revealed that these succinic acids, clustered with alcohol, ether, phenol, and ketone functional groups, could form polydentate, chelate complexes of both inner-sphere and outer-sphere types that involved a major portion of the molecular structure. Both aromatic and aliphatic substituted succinic acid structures can be derived from the β-keto adipate pathway of ring biodegradation (Crawford, 1981) as illustrated below for a model humic acid:



Dissolved organic matter structures derived from different plant and microbial precursors in various environments (Leenheer, 1994) converged to similar structures with clustered carboxyl groups despite different pathways of oxidative biotic and abiotic degradation.

4. Conclusions

Oxidative diagenesis of plant and microbial precursors of natural organic matter results in clustered carboxyl, hydroxyl, and ketone group structures that form strong metal-binding groups.

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KINETICS AND MECHANISMS OF LEAD SORPTION BY IRON OXIDES FORMED UNDER THE INFULENCE OF CITRIC ACID

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1. Introduction

The chemical reactions of Pb sorption on pure goethite were reported by Hayes (1987). However, various ions and molecules are present during the formation of Fe oxides and can be incorporated into their structures (Cornell and Schwertmann, 1996). Thus, pure Fe oxides rarely exist in natural environments. The presence of citric acid, which is very common in soils at the concentration of 10^{-5} M (Robert and Berthelin, 1986), during the formation of Fe oxides greatly influenced their surface properties through fundamental structural modifications (Liu and Huang, 1998). The objective of this study was to determine the impact of the surface properties of Fe oxides formed under the influence of citric acid on the kinetics and mechanisms of Pb sorption on these Fe oxides by using pressure-jump relaxation spectrometry.

2. Materials and Methods

Iron oxides were formed in a 0.01 M ferrous perchlorate at pH 6.00±0.05 and 23.5 °C both in the absence and presence of citric acid. The initial citrate/Fe(II) molar ratios (MR) were 0 and 0.001. The Pb sorption experiments included equilibrium (adsorption isotherms) and kinetic (relaxation) studies. In both of the studies, the Fe oxide concentration was 15 g L^{-1} and the initial concentration of Pb as $Pb(NO_3)_2$ was 3.0 mM and 2.5 mM for the Fe oxides formed at the initial citrate/Fe(II) MRs of 0 and 0.001, respectively. The pH of the suspensions was adjusted to 2-6 by adding different volumes of 0.10 M NaOH or HNO_3 solution. The ionic strength of the equilibrating solutions was 0.015.

3. Results and Discussion

The adsorption edge of Pb on these two Fe oxides can be described by the modified triple layer model (TLM) using the FITEQL program (Herbelin and Westall, 1994). Two relaxation times observed in the Pb and Fe oxide suspensions at one pH value indicate that Pb adsorption on the Fe oxides formed both in the absence and in the presence of citrate ligands at the initial citrate/Fe(II) MR of 0.001 consisted of two elementary reactions.

The results revealed that the mechanisms of Pb sorption at the goethite/water interface derived by Hayes (1987) were also acceptable for the Pb sorption in the two Fe oxide/water systems in this study. However, the intrinsic forward rate and equilibrium constants (k_1^{int}, k_2^{int}, K_1^{int}, K_2^{int}) for the two reactions of Pb sorption by the Fe oxides formed in the absence of citrate were significantly higher than those in the presence of citrate at the MR of 0.001 (Table 1). This is due to the improvement of the crystallinity of Fe oxides caused by the presence of citrate at the MR of 0.001 during their formation through catalysis and the subsequent decrease in their specific surface area (Liu and Huang, 1998).

Table 1. The intrinsic rate constants and equilibrium constants for lead sorption on the Fe oxides formed at the initial citrate/Fe(II) MRs of 0 and 0.001

	Initial citrate/Fe(II) MR		$LSD_{0.05}$	$LSD_{0.01}$
	0	0.001		
k_1^{int} ($M^{-1}s^{-1}$)	17.6 x 10^6	7.38 x 10^6	3.04 x 10^6	7.01 x 10^6
k_{-1}^{int} ($M^{-1}s^{-1}$)	1.03 x 10^5	1.09 x 10^5	4.24 x 10^4	9.78 x 10^4
K_1^{int}	17 x 10^1	6.8 x 10^1	4.8 x 10^1	11 x 10^1
k_2^{int} ($M^{-1}s^{-1}$)	8.60 x 10^6	5.26 x 10^6	2.89 x 10^5	6.66 x 10^5
k_{-2}^{int} ($M^{-1}s^{-1}$)	7.11 x 10^4	8.48 x 10^4	4.06 x 10^3	9.36 x 10^3
K_2^{int}	12.1 x 10^1	6.21 x 10^1	9.27	21.4

Compared to the Fe oxides formed in the absence of citrate ligands, the intrinsic backward rate constant (k_{-2}^{int}) for the second reaction of Pb sorption by the Fe oxides formed at the MR of 0.001 was significantly higher than that at the MR of 0. The Pb desorption on the Fe oxides in the second reaction involves the breaking of bond of surface coordinated Pb and nitrate ion-pair. The nitrate inhibits the breaking of Pb surface coordination bond by shielding the Pb^{2+} ion from attack by a proton (Hayes, 1987). Compared to the Fe oxides formed at the MR of 0, the lower point of zero salt effect of Fe oxides formed at the MR of 0.001 and more net negative charges at the same pH (Liu and Huang, 1998) caused less coordination of nitrate ions with Pb on the surface. Therefore, the nitrate ions less effectively shielded the Pb^{2+} ion from attack by a proton in the Fe oxides formed at the MR of 0.001.

4. Conclusions

The presence of citric acid at the concentration of 10^{-5} M during the formation of Fe oxides significantly influenced the intrinsic rate and equilibrium constants of Pb sorption at the Fe oxide/water interface through fundamental structural modifications and the subsequent alteration of their surface properties.

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DOC INDUCED RELEASE OF CADMIUM AND COPPER FROM SOIL

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1. Introduction

Afforestation of arable soils introduce formation of O-horizons and DOC fractions (dissolved organic carbon) with chemical composition different from DOC in arable soils (STROBEL et al. 1999). The DOC components mobilise metal cations due to desorption and weathering by complexation of metal cations at the mineral surface (DREVER 1994; KELLY et al. 1998; RAULUND-RASMUSSEN et al. 1998). The release of cadmium and copper from soils are effected by DOC and other complexing agents.

The aim of this study was to determine the effect of the DOC and pH conditions introduced by afforestation on the release of cadmium and copper in the former intensively cultivated arable soils.

2. Materials and Methods

A pair of A-horizon soil samples were collected from clayey till at Christianssæde in Denmark. One sample from a Norway spruce (*Picea abies*) stand planted on arable soil in 1963 and the other from an intensively cultivated arable soil next to the forest.

Soil solution DOC was obtained by centrifugation of forest floor litter (Oe-horizon) under Norway spruce. The DOC solution was passed a 0.45 μm Sartorius RC filter and cation exchanged triply to bring it into the protonated form.

For the desorption experiments 5.0 gram soil was reacted with an influent solution (pH adjusted DOC solution) in a magnetically stirred Plexiglas continuous flow cell with inlet at the bottom and outlet from the top end to a fraction collector. Influent solution series with two pH levels (3.7 - 5.1) at two DOC concentrations (0 - 5 mM) were prepared. The effluents were analysed for cadmium and copper by graphite furnace AAS.

3. Results and Discussion

Results for the release of cadmium and copper from the clayey till soil ascribe desorption effect to both DOC and pH. The release rates calculated as the linear slope of the accumulated release curves were all constant (Fig. 1). The pH induced (no DOC) cadmium release rate was 3 times faster at pH 3.7 than at pH 5.0 and the addition of DOC increased the release rates 1.2 and 3 times, respectively. The relative DOC effect on cadmium desorption was low at pH 3.7 and higher at pH 5.1, but the absolute DOC effect on the cadmium release rate was higher at low pH (Fig. 1).

The pH induced copper release rate was 2.2 times faster at pH 3.7 than at pH 5.0. Addition of 5 mM DOC doubled the copper release rate at pH 3.7 whereas at pH 5.1 the apparent copper release rate was reduced to 0.45 times the rate without DOC in the system. The complexation of copper by colloidal organic matter might increase at higher pH thus retaining copper in

flocculated humic substances in the immobile fraction.

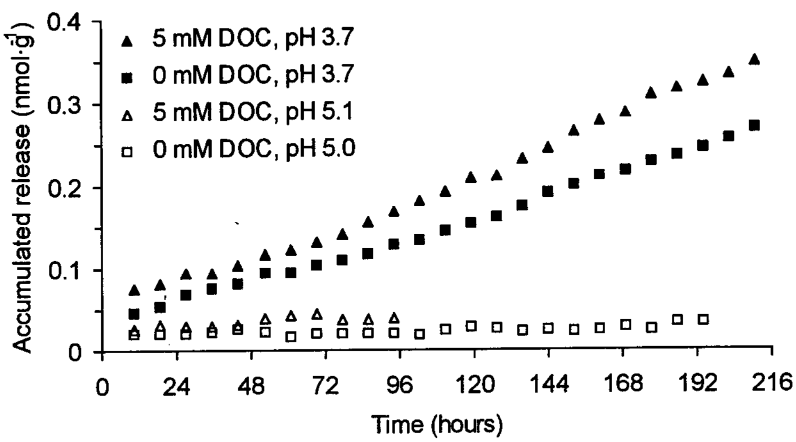

Figure 1 Accumulated cadmium release at pH 3.7 and 5.1 from Christianssæde arable soil with and without 5 mM DOC in the influent solution.

4. Conclusions

DOC increased the mobility of cadmium and copper at low pH whereas at higher pH only the cadmium release rate was increased by DOC. At pH 5.1 the apparent copper release rate was reduced compared to the experiments without DOC, probably due to stronger complexation by humic substances and thus flocculated in the soil system.

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CU(II) COMPLEXATION CAPACITY OF LITTER LEACHATES AND SOIL SOLUTIONS IN ACID FOREST SOILS

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1. Introduction

In some acid forest soils, surface podzolization involves the migration of metal-humus complexes only at short distances. The process is hardly detectable because the spodic material is not easily distinguished from the surface hemiorganic horizons. The measurement of the ability of dissolved organic matter (DOM) to complex metals could be useful for a further diagnostic. Although Cu(II) complexation capacity (Cu-CC) and Al-CC of DOM may differ (LUSTER et al., 1996), the former could be used to get a rough estimate of the latter in order to compare the podzolization potential of litter leachates and soil solutions.

2. Materials and Methods

In a loess-derived toposequence (BRAHY and DELVAUX, 1997), surface podzolisation has been diagnosed in a Dystric Cambisol (P4) with *mor* humus, on the basis of Fe, Al selective dissolution data. Vegetation is a broad-leaf forest where the dominant species is beech (*Fagus sylvatica*). We reconstituted litters involving O and OAh horizons in small columns. Litter leachates were collected after percolation of a water depth of 36 mm using a throughfall solution. Soil solutions were collected in-situ by low-tension lysimeters inserted at different depths following horizon morphology (figure 2). Cu-CC was measured using the methodology of Town and Powell (1993) in both litter leachates and soil solutions. Dissolved Organic Carbon (DOC) and concentrations in cations and inorganic anions were also determined.

3. Results and Discussion

Results are illustrated for two *Dystric Cambisols* P3 and P4, with a dystric moder and a fibrimor, respectively. In the litter leachates (figure 1), Cu-CC is higher in P4 relatively to P3. On the basis of the distinct stability of metal-humus complexes, Cu-CC is thought to estimate the residual complexation capacity for Al and Fe. The litter leachates of P4 therefore account for a higher ability to complex additional amount of Al and Fe. The similarities in concentrations of total Fe and Al suggest that differences in concentrations of saturated complexing sites are most likely very small respectively to differences in concentration of residual complexing sites. Therefore, total concentration in complexing sites (total complexation capacity) could also be higher in P4 litter leachates. These litter leachates are also characterised by higher Cu-CC/DOC ratios: 1,11 $.10^{-2} \pm 3{,}3\ .10^{-3}$ (n=3) versus 4,1 $.10^{-3} \pm 2{,}6\ .10^{-3}$ (n=3) for litter leachates of P3.

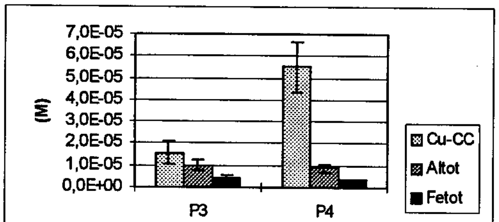

Figure 1: Cu-CC, Fe and Al total concentrations in litter leachates: means and standard errors (n=3).

In the soil solutions, the Cu-CC is slightly higher in surface horizons of P4 (figure 2), but the Cu-CC/DOC ratio is clearly higher in these horizons and is very small in subsequent horizon (figure 3). The higher density of residual complexing sites of DOM in P4 superficial horizon solutions (Ah and AB1) and P4 litter leachates suggests a higher mobility of DOM a higher potential for podzolization.

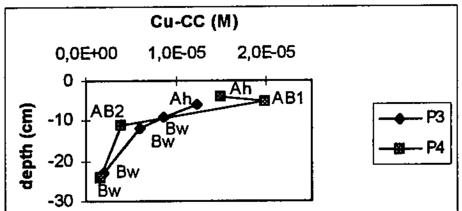

Figure 2: Depth distribution of Cu-CC measured in soil solutions

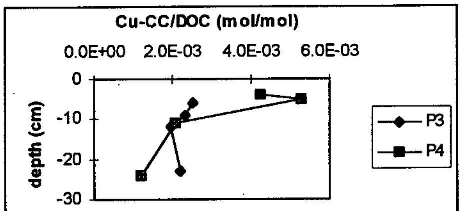

Figure 3: Depth distribution of Cu-CC/DOC in soil solutions.

The presence of soluble organics in surface horizons of podzols is associated to an anionic charge deficit. In litter leachates, a positive correlation is observed between the Cu-CC (y) and the anionic charge deficit (x): $y = 0{,}12\ x - 5.10^{-5}$ ($r^2 = 0.82$, n=12). The linear equation (slope << 1 and $x_0 >> 0$) indicates that part of the organic acids functions does not contribute to complexation capacity and more than one function is involved in the complexation of one cation, suggesting the existence of chelate-type relations.

4. Conclusions

The Cu-CC of DOM in soil solutions have been used to compare podzolization potential of these solutions. A higher potential has been associated to litter leachates and soil solutions from upper horizons of the soil exhibiting surface podzolization characters.

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COMPLEXATION AND COMPETITIVE SORPTION OF STRONTIUM BY HUMIC ACID ON DIFFERENT BACKFILL CLAY MINERALS

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1. Introduction

Bentonite clay is one of the backfill materials designed to prevent the leakage of nuclear waste repositories due to its excellent swelling property. However, radionuclides may permeate through such an engineered barrier and release into the soils. Strontium (Sr) is one of the radionuclides of most concern that accumulated for decades in Taiwan. Researches have reported that strontium can be strongly sorbed onto the mineral surface of soil (ADELEYS and CLAY, 1994). Recent work suggested that radionuclides can undergo complexation with humic substance and transport through soils without significant retardation (TEGEN and DORR, 1996). However, complexation of strontium with humic substance on different buffered clay minerals is not well understood. Also, the competitive behavior between strontium and other radionuclides in organic rich system still remains unclear.

The objective of this study was to evaluate the effect of humic acid on the sorption behaviors of strontium on different clay minerals. Two sets of sorption systems, the single-ion system and multi-ions system, were conducted to elucidate the competition behavior of strontium. Also, pH effect on the complexation of strontium is discussed.

2. Materials and Methods

Three buffer materials, Jinsin bentonite, montmorillonite and kaolinite, were selected as the target clay minerals. All of the clays were sieved with 325-mesh sieve and heated to 550°C for 2 hours to remove organic content. Batch sorption experiments were performed by adding 2.0 g of clay minerals to 40 mL buffer solutions in 50 mL centrifuge tubes. Strontium-85, acting as tracer, was mixed with strontium-88 at a ratio of 1:10 to give the concentrations of 0.01 mM to 2.5 mM. The concentrations of humic acids were in the range of 50 mg/L to 200 mg/L. The pH values of solutions were adjusted with phosphate buffer solutions to yield pH 4 to pH12. The series of solutions were reacted for 24 hours at 150 rpm and 25°C. After centrifugation (5500 rpm for 10 min), supernatants were transferred into the PTFE vials and were analyzed by NaI or HPGe detector.

3. Results and Discussion

Figure 1 illustrates the sorption of strontium on different clay minerals without the addition of humic acid. The sorption capacity of strontium was highest for montmorillonite-amended system, whereas a 4-fold decrease in sorption capacity of Sr in kaolinite-amended system was demonstrated. Bentonite constitutes of 80% montmorillonite and 15% kaolinite and its sorption capacity reached 1.68μmole/g, which was 80% of sorption capacity of Sr by montmorillonite. Linear relationships between the sorbed Sr and the equilibrium concentrations in liquid phases were observed, showing that ion exchange mechanism predominated when the liquid concentration of Sr was lower than 0.3 mM. Increasing concentration could change the sorption isotherm and the BET isotherms can be fit at higher equilibrium strontium concentration. Also, different sorption isotherms and sorption capacities were observed for the competitive system.

The sorption capacity of Sr decreased 60% and the isotherm was fit by Langmuirian expression in multi-ions systems.
Figure 2 illustrates the effect of organic content on the sorption of Sr. Increasing the concentrations of humic acid lowered the sorption of Sr in bentonite- and montmorillonite-amended systems, whereas the sorption of Sr by kaolinite increased concomitant with the increasing concentration of humic acid. It can be speculated that the added humic acid can be sorbed or precipitated onto the surface of kaolinite and undergo complexation reaction with Sr, thereby increasing the sorption capacity of strontium.
The results of pH value on the sorption of Sr showed that increasing pH value could enhance the sorption capacities of Sr on different clays, especially when the pH value was higher than 7. The apparent precipitation of Sr under alkali environment resulted in the rapidly increasing sorption capacities. It is obviously that little precipitation effect occurred under acidic environment. The activation of mineral surface sorption sites by the increased OH^- ions can explain the low sorption capacity of strontium under acidic conditions.

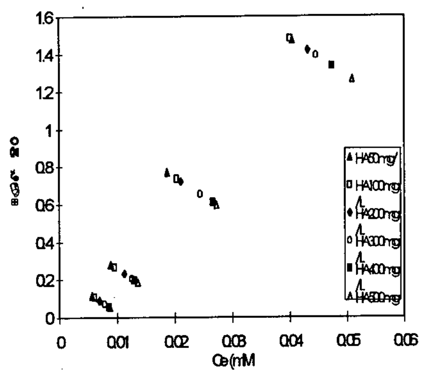

Figure 1. Sorption of strontium on different clay minerals without the addition of humic acid.

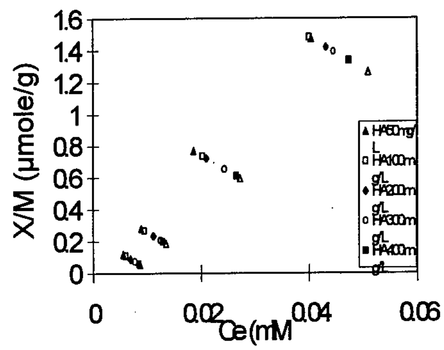

Figure 2. Effect of organic content on the sorption of strontium by Jinsin bentonite.

4. Conclusions

Results obtained in this study indicate that the organic content and pH value are of importance influencing the sorption characterization of strontium. The addition of humic acid could change the sorption capacities and isotherms of Sr. Increasing pH value could enhance the sorption capacities of strontium on different clays, which was attributed to precipitation of strontium and activation of mineral surface sorption sites.

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TIME-DEPENDENT TRANSFORMATIONS OF HEAVY METAL BINDING ONTO ORGANOGENIC MATTERS

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1. Introduction

Despite long-lasting interest in the mechanism of metal binding onto natural organic matter, the common viewpoint on this subject is still far from being reached. There appears, however, to be an agreement on the issue of the kinetics of metal sorption onto organic matter: the rate of sorption is generally considered to be rapid, from several seconds to 1 hour (Allen, 1996).

Main objective of these studies is developing knowledge on the mechanisms of metal binding/release onto natural organic matter. The present research has been focused on the timingand mode of organic adsorbent/metal ion interaction as factors affecting sorption capacity and binding strength of organic matter for metals, which determines their susceptibility to remobilization.

2. Materials and Methods

Peat was selected as an almost pure humic-rich organic material. Short-term batch and long-term column experiments on Zn^{2+}, Cd^{2+}, Cu^{2+} and Cr^{3+} binding onto homogenized peat matter from monometallic solution at pH 4.0. The sequential extraction (KERSTEN and FORSTNER, 1988) was applied to differentiate "pools" of metal enrichment in the matrix of increasing binding strength and decreasing vulnerability to remobilization. Parallel batch sorption experiments on these compounds extracted from peat matter were also conducted.

3. Results and Discussion

Assuming that enrichment in F1(EXC) fraction reflects the quantity of metals bound electrostatically and in F4(MRO) fraction due to chelating complex formation in "soluble organic" compounds, while the metal enrichment in F5 (OM) fraction of the highest binding strength is associated with "insoluble organic" residue comprising humins, cellulose and lignin, the role of these mechanisms appeared to be of a diverse importance with respect to studied metals and time-dependent conditions of sorption (Fig.1A,B)

Under short-term batch conditions, the capacity of the studied peat matrices to bind metals (in mass units) followed the descending order: Cr>Cu>Cd>Zn. The fractionation of Zn and Cd bound in the batch experiments showed predominance of the most labile fractions F0(PS)+F1(EXC)+F2(CARB). Cu was uniformly distributed in the fractions. For Cr, a predominant enrichment in the most strongly bound F5(OM) phase ("insoluble organic) occurred (Fig. 1 A).

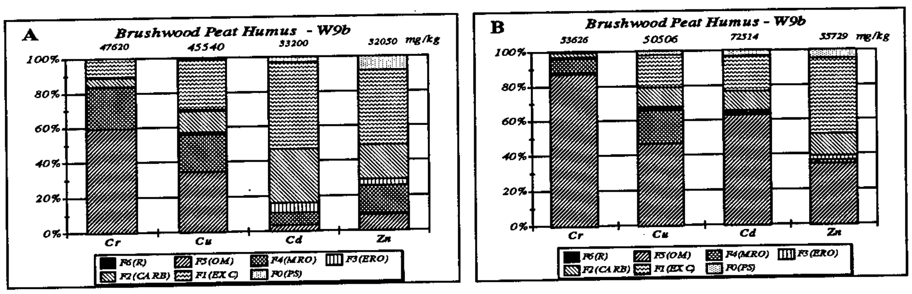

Figure.1: Sequential fractionation of Cr^{3+}, Cu^{2+}, $Cd^{2+}$and Zn^{2+} bound from monometallic solution short-term batch (A) and long-term (B) conditions at c_0= 5000 mgMe dm^{-3} and pH 4.0 onto Brushwood Peat Humus according to the increasing binding strength. Fractions: F0(PS) – pore solution; F1(EXC) – most labile; F2(CARB) – labile; F3(ERO) – easilyextracted; F4(MRO) – moderatelyextracted; F5(OM) strongly bound ("insoluble organic")

Under long-term dynamic conditions, the total binding capacity (in mass units) followed the order Cd>Zn>Cr><Cu. Chemical fractionation of metals showed virtual difference compared to batch systems (Fig. 1B), which consisted mainly in a high metal enrichment in the "insoluble organic" fraction F5(OM) It occurred either independently, which resulted in high increase of the sorption capacity (Cd, Zn) or simultaneously with the decrease of a metal enrichment in the "soluble organic" fractions (Cr, Cu). This suggests the transformations of binding mechanisms from the weaker to the stronger ones. A significant increase of metal resistance to desorption compared to batch process confirms this conclusion.
It was found that humic acids (HA) participate in Zn and Cr sorption as one of the major components of the total sorption capacity. For Cu, their role seems to be less significant and for Cd negligible.

4. Conclusions
The observed transformations of metal ion sorption capacity and binding strength of peat, dependent upon the mode and duration of contact with adsorbate show that kinetics and mechanism of metal binding on the natural organogenic matter is of a more complex nature than has been generally assumed. The sorption pattern appears to consist of two groups of reactions of different kinetics. Long-term reactions evolve sorption of a strong type, results in a high rate of metal enrichment in insoluble organic fraction. The sorption process involves a variety of sorption sites in the organogenic matrix, of which complexing metal ions by HA was found to be only partially and differentially responsible for metal binding. This challenges the theory of the primary responsibility of HA for metal binding onto organogenic sorbents like peat and suggests the need of identification of other sorption sites of importance.

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CHARACTERISTICS OF COMPOST-DERIVED HUMIC SUBSTANCES AND THEIR REACTIONS WITH PB, CU, CD AND ZN

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1. Introduction

The extension of organic farming and sustainable agriculture has led to increasing applications of organic fertilizers. Organic fertilizers of composts contain a substantial amount of organic matter, subsequently containing a significant amount of humic substances [DEIANA et al. (1990); TZENG et al. (1996)]. In this study, we investigated the average conditional concentration quotients of the complexes formed by reacting heavy metals with humic substances of compost in the suspension of heavy metal-humic acid and the solution of heavy metal-fulvic acid systems at initial pH values of 4.00 and 6.50. In addition, the sequence of the bonding strength between various humic substances of compost and heavy metals was also ranked.

2. Materials and Methods

A commercialized swine compost was selected to perform the experiment. According to the method recommended by the International Humic Substances Society (IHSS), the humic acid (HA) and fluvic acid (FA) were extracted from the compost and purified [AIKEN (1985); HAYES (1985); LEENHEER (1985); SWIFT (1985)]. The solution of purified fulvic acid was then dialyzed against deionized water by using a dialysis tubing with a molecular-weight cutoff of 1,000 to separate the fraction of FA (MW < 1000) from that of FA (MW > 1000) [WANG and HUANG (1987)]. The freeze-dried humic substances were subjected to the determination of composition by elemental analysis and that of functional groups by Fourier transform infrared spectroscopy (FTIR) and solid-state ^{13}C nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning (CPMAS ^{13}C NMR). Titration was also performed to determine the total acidity values [SCHNITZER and KHAN (1972)]. The concentrations of the free ions of lead, copper, cadmium and zinc in the reaction systems of heavy metal-humic acid suspensions and heavy metal-fulvic acid solutions were measured by anodic stripping voltammetry (ASV) [ZEN et al. (1995)] to calculate the average conditional concentration quotients ($\overline{K}^*$) of the reaction of humic acid and fulvic acids of the compost with heavy metals [PERDUE (1991)].

3. Results and Discussion

^{13}C nuclear magnetic resonance spectra indicate that carboxyl contents of fulvic acids of the compost surpass that of humic acid. The sequence of total acidity values of humic substances of the compost was FA (MW < 1000) > FA (MW > 1000) > HA (MW >1000). Table 1 shows that the sequence of the average conditional concentration quotients of the formed complexes from the reaction of humic substances of the compost with heavy metals was FA (MW < 1000) > FA (MW > 1000) > HA (MW > 1000). In addition, the average conditional concentration quotients of the reaction systems were higher at the initial pH of 6.50 than at the initial pH of 4.00. This is attributed to higher dissociation of total functional group acidity of the humic substances at pH 6.50 than at pH 4.00 [SCHNITZER and KHAN (1972); SPOSITO et al. (1982); PERDUE

(1991)]. Based on different kinds of heavy metals, the sequence of the reaction of humic substances of compost with four kinds of heavy metals was lead > copper > cadmium > zinc (Table 1), displaying large discrepancies among the reactions of various heavy metals with humic substances of a kind of compost. Our study revealed that the sequence of the average conditional concentration quotients of the reaction of humic substances of the compost with lead, copper, cadmium and zinc met the reported sequence of adsorption and affinity studies.

4. Conclusions

Both the characteristics of humic substances and chemical behavior of heavy metals substantially affect the bonding strength between humic substances and heavy metals. These may thus affect the mobility and bioavailability of heavy metals in soil environment.

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Table 1: The average conditional concentration quotients obtained from the reactions of 7.98×10^{-5} N of binding site of HA (MW > 1000) and of 1.00×10^{-5} N of binding site of FA (MW > 1000) or FA (MW < 1000) derived from the compost with the same equivalent concentrations of Pb, Cu, Cd or Zn at initial pH of 4.00 and 6.50.

Heavy metal	Initial pH	Average conditional concentration quotient, $\overline{K^*}$		
		HA (MW > 1000)	FA (MW > 1000)	FA (MW < 1000)
		N^{-1}		
Pb	4.00	1.16×10^4	7.24×10^4	1.37×10^5
	6.50	3.16×10^4	9.91×10^4	2.33×10^5
Cu	4.00	3.09×10^3	2.24×10^4	3.33×10^4
	6.50	6.94×10^3	3.11×10^4	4.07×10^4
Cd	4.00	4.96×10^3	2.42×10^4	3.21×10^4
	6.50	5.73×10^3	3.29×10^4	3.84×10^4
Zn	4.00	7.38×10^2	5.38×10^3	8.89×10^3
	6.50	2.48×10^3	1.53×10^4	1.24×10^4

INFLUENCE OF HUMIC ACID ON THE SORPTION OF ARSENATE ON KAOLINITE

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1. Introduction

The toxic effect of arsenate to men has been known for a long time. To be able to predict the fate of arsenate in the environment, it is essential to study the sorption of arsenate by soil minerals. As clay minerals are strong sorbents for anions and cations, the sorption behavior of different clay minerals has been subject of numerous studies. In the environment, however, mineral surfaces are often covered by organic compounds like e.g. humic acids. Information about the influence of organic coverage of minerals on the sorption of contaminants is very rare. The objectives of this study were to investigate (i) the sorption of arsenate to kaolinite as a function of pH and ionic strength, and (ii) the effect of humic acid on the sorption of arsenate to colloidal kaolinite.

2. Materials and Methods

Preparation of colloidal Na-Kaolinite

Georgia kaolinite (KGa-2) was obtained by The Clay Minerals Society. The fraction less than 1 µm in diameter was gained by repeated centrifugation. Colloidal kaolinite was saturated with sodium by repeated washing with a 1 M NaCl solution. Excess salts were removed by washing with deionized water. Kaolinite particles were characterized with transmission electron spectroscopy (TEM), N2-BET surface area measurements, and powder X-ray diffraction analysis (XRD).

Preparation of Humic Acid

Humic acid was extracted from a humified organic horizon (H) of a Humic Gleysol, using standard extraction and purification procedures as recommended by the International Humic Substance Society. The ash-free humic acid was dialyzed for two weeks against deionized water containing a mixed-bed ion exchange resin, followed by dialysis against deionized water for one month. The purified humic acid was stored as solution, containing 2.269 mg C per liter, in the dark at 4 °C.

Potentiometric titrations

Charging behavior of Na-kaolinite colloids and humic acid was determined by acid-base titrations at NaNO3 electrolyte concentrations ranging from 0.01 to 0.3 M using an automatic titration set-up.

Arsenate adsorption experiments

The pH-dependent adsorption of arsenate to kaolinite was investigated with batch experiments. Solid concentrations and total arsenate concentrations ranged from 0.49 to 4.9 g/L and 1 to 100 *µM*, respectively. The pH values were adjusted by adding either HNO_3 or NaOH. All experiments were performed in $NaNO_3$ background electrolyte. All samples equilibrated under

nitrogen gas for 21 h at 25 °C were filtered with 0.1-μm membrane filters prior to analysis for arsenate with atomic absorption spectroscopy.
Competitive sorption experiments with humic acid and arsenate were carried out at constant kaolinite and arsenate concentrations of 5.15 g/L and 19.5 μM, respectively. The maximum humic acid concentration was 32 mg C/L. The preparation procedure of samples containing humic acid was similar to the one without humic acid.
Humic acid concentrations were measured by a total carbon analyzer (TOC-5000, Shimadzu).

3. Results and Discussion

Kaolinite and humic acids have a charging behavior that is strongly dependent on ionic strength. By fitting the titration data with a 2-pK Basic Stern model a point of zero charge of the edge surfaces (PZC_{edge}) was found to be at pH 5.3 ± 0.1.
The adsorption of arsenate is strongly dependent on pH and ionic strength. Significant amounts of arsenate were sorbed at pH > PZC_{edge}, decreasing with increasing pH. Effects of ionic strength on arsenate adsorption were only observed at pH < PZC_{edge}. These results suggest that arsenate is sorbed both ways, specificly and unspecificly, by kaolinite.
Humic acid adsorption decreased with increasing pH. In the presence of humic acid a decrease of arsenate adsorption was observed (Fig. 1). Humic acid competes for anion sorption with arsenate a similar way phosphate does, when exceeding arsenate concentrations by far (MANNING and GOLDBERG (1996)).

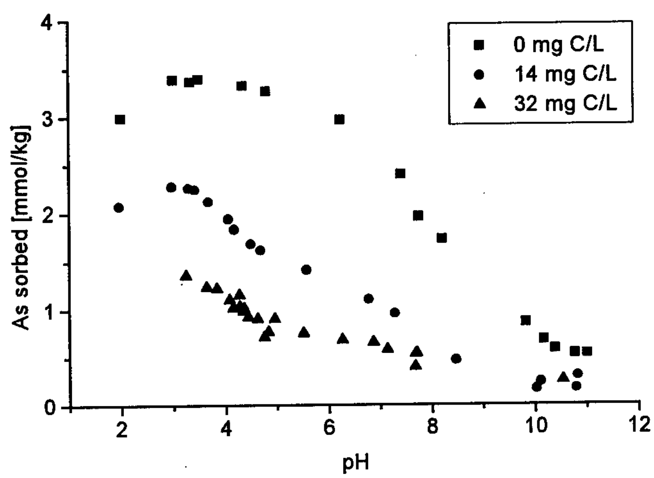

Figure 1: Influence of humic acid to arsenate sorption on kaolinite. Kaolinite and total arsenate concentrations were 5.15 g/L and 19.5 μM, respectively.

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HUMIC ACIDS AS NATURAL LIGANDS, THEIR ACID-BASE PROPERTIES AND INTERACTIONS WITH TRACE METALS

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1. Introduction

Humic acids (HA) are natural compounds which form in different geochemical environments and contribute greatly to the migration cycles of transition metals due to formation of stable metal-humic complexes. The properties of humic acids as natural ligands are due to a large number of functional groups with different acidic strengths. Complexing capacity of HA depends on the degree of ionization of these groups which is pH-dependent. In our previous work (Orlov *et al.* 1998) we have obtained stability constants of fractionated HA with Cd and Zn proceeding from the molar concentration of the ligand and HA dissociation constants taken from the literature (Stevenson, 1976). In this work we use another calculation technique based on considering the content of functional groups, deprotonated at a definite pH. Potentiometric titrations were performed for this purpose.

2. Materials and Methods

1) HA preparation. Humic acid extracted by 0.1 M NaOH from the paste of brown coal HA (HAC) and peat HA purchased from "Merck" (HAP) were studied. Ash content of the samples was < 3% after purification. Fractions with molecular weights 4700 Da (HAP) and 5000 Da (HAC) were obtained by gel-chromatography. *2) Potentiometric titration.* HA were diluted in 0,05 M NaOH (2mg/ml) and then reverse (pH 11.5-2.5; titrant 0,1 M HCl) and after that immediately forward (pH 2.5-11.5; titrant 0,1 M NaOH) titrations were performed at 25° C, ionic strength 0,1 (KCl), under permanent N_2 flow. HA titration start and end points were found by Gran's analysis, equivalence points - as $\Delta pH/\Delta V_{max}$ on first derivative curves of titre volume vs. pH, dissociation constants (pK_a) - using Henderson-Hasselbach equation. *3) Stability constants determination.* Shubert's ion-exchange equilibrium method with using of ^{65}Zn and ^{109}Cd isotopes was used for this purpose. Stability constants were obtained at ionic strength 0,1 (KNO_3), pH 4 and 8, 25° C and a metal:ligand molar ratio of $5 \cdot 10^{-7} / 5 \cdot 10^{-6}$ to $5 \cdot 10^{-7} / 5 \cdot 10^{-5}$.

3. Results and Discussion.

The shapes of titration curves were similar for the humic acids studied with two distinct peaks of $\Delta pH/\Delta V_{max}$ for reverse titration and one for forward titration. Start and end points of HA reverse titration were about 10.5 and 3.5 respectively and of forward titration - 3.5 and 10.0 respectively. In the region of pH values over 6,0 hysteresis between forward and reverse titration curves was observed. We assumed that in the pH range 3.5-7.0 carboxylic groups are being titrated (pK_{a1}), over 8.5-9.0 - phenolic (pK_{a3}), and the intermediate pH range of 7.0-8.5 is overlap of the two groups mentioned and NH_2 - groups (pK_{a2}). The dissociation constants for HAP (HAC) reverse titration are pK_{a1} 4.7 (4.8); pK_{a2} 8.1(8.0); pK_{a3} 9.8 (9.8); for forward titration: pK_{a1} 4.9 (5.1); pK_{a3} 9.3 (9.2). pK_{a1} values are in good agreement with those obtained by F.Stevenson (1976) whose data we used in our previous work (Orlov *et al.* 1998).

According to Shubert's method it was assumed that complexes of only one type form in the solution, i.e. : $Me^{2+}+nL^{x-}\leftrightarrow MeL_n^{(x-n)-}$ and $\beta_c = [MeL_n^{(x-n)-}]/\{[Me^{2+}]\cdot[L^{x-}]^n\}$, where L is ligand ($HA_p$ or HA_c); Me is Zn^{2+} or Cd^{2+}; β_c is pH-independent stability constant.

Ligand concentration can be expressed in terms of 1) molar concentration of HA with considering the competitive reaction of carboxilic groups protonation (pK_{a1}); 2) the number of functional groups ionised at a definite pH (4 or 8) which can be found from potentiometric titration curve.

The data obtained strongly depends on the calculation technique used (Table 1), but the overall tendency rests the same - with increasing pH log β_c increases and complexes with a L / Me ratio

Table 1: tability constants and structure (L/Me ratio, n) of metal-humic acids complexes. *-refers to calculation technique (1); ** - refers to calculation technique (2).

Metal	Ligand	pH 4		pH 8	
		n	lg β_c	n	lg β_c
Cd	HAP	1.2	6.8* [4,9]**	1.5	8,4 [6,6]
Cd	HAC	1.4	7.4 [5,3]	1.9	10,6 [8,2]
Zn	HAP	1.0	4.6 [3,3]	1.3	7,5 [5,9]
Zn	HAC	1.0	5.0 [3,5]	1.4	8,0 [5,6]

2:1 form. This seems to contradict the fact that at high pH many of acidic groups are ionized (according to our data at pH 4 there are 2-3 COO^- groups per HA molecule and at pH 8 there are 16-18 COO^- groups), and repulsive forces between them should decrease complex stability and prevent formation of binary complexes. However existence of such structures have been already shown by Stevenson (1977), Orlov *et al.* (1988), etc. and this phenomenon may occur because with increasing pH more active sites of HA are deprotonated and available for binding with metal ions. One of the reasons for high stability of metal-HA complexes may be conformational changes of HA molecule as a result of which metal ion can be fixed in its inner volume and interaction with ions from solution is limited. It is important for metal geochemistry since in this case it is the base-acidic properties of humic acids that control migration of metal ions.

4. Conclusions

1. Stability constants values highly depend on the method of calculation of ligand concentration.
2. Stability of HA-metal complexes appears to be controlled by HA ionization process and conformation changes of macroligands.

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ORGANO-METALLIC COMPLEXES WITH MAJOR AND MINOR ELEMENTS IN SOME ANDIC SOILS FROM THE NORTH-WESTERN CARPATHIANS (ROMANIA)

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1. Introduction

This paper gives some results from a study of humus composition and organo-metallic complexes in 11 soil profiles, ranging from Haplic Andosols to Andi-Dystric Cambisols from the Ignis-Oas Mountains, located in the northern part of the Romanian Eastern Carpathians.
In order to illustrate the results obtained for organo-metallic complexes two soils with relatively different properties have been selected:
Soil I : A Haplic Andosol considerably influenced by deposition of heavy metals and strong acid from ore-processing industries.
Soil II : An Andi-Dystric Cambisol with minor influence from air pollution.

2. Materials and Methods

The total organo-metallic complexes were extracted with sodium pyrophosphate, pH 10. This reagent was chosen because of its high extraction capacity and selective complexation (Alexandrova, 1960, Bascomb, 1968, Schnitzer and Schuppli, 1989).
The humic and fulvic fractions were further separated after adjustment to pH 1.5 with sulphuric acid. After treatment with aqua regia, the concentrations of Al, Fe, Mn, Cr, Cu, Zn Cd and Pb and the base cations K, Mg and Ca associated with humic matter and fulvic fraction were determined by atomic absorption spectrometry and the distributions between fulvic and humic-bound complexes were calculated.
Total acid soluble metal fractions were obtained by heating a 2-gram soil sample overnight with 10 ml of 14 M HNO_3. After removal of undissolved mineral matter and appropriate dilution the extracted metal concentrations were determined by atomic absorption spectrometry.

3. Results and Discussion

In the Haplic Andosols the fraction of organic carbon extracted with pyrophosphate was significantly higher than in the Andi-Dystric Cambisols. Except for Ca all the studied metals were more abundant in the fulvic than in the humic fraction. Some of the profiles exhibited considerable surface contamination of Pb and Cd due to emission from metal smelters of the region. Such contamination was not evident for other heavy metals such as Cr, Cu, and Mn.
By comparison of the pyrophosphate-extracted and the HNO_3-soluble fraction of the elements, the observed ratios ranged as follows:

Soil I: Cu > Pb, Cr > Ca, Cd > Al, Fe, Mn, K > Mg.

Soil II: Cu > Pb > Ca >> K, Mg > Cr, Mn, Zn, Cd > Al, Fe.

This ranking was almost identical regardless of whether the whole soil profile or only the near-surface horizons were considered. We believe that the higher ratios observed for Cu and Pb at least in part are explained by a higher degree of complexation of these metals than of the others. This is in accordance with the literature (Schnitzer, 1966; Schnitzer and Skinner, 1966; Schnitzer and Kerndorff, 1981). It is conceivable that at least some of the pyrophosphate extracted elements may have occurred in other forms at pH 10 than just as organo-metallic complexes. It does not appear very reasonable, for example, than monovalent K^+ cation should posses a higher complexing power than some of the divalent cations studied. In this and perhaps also other cases, it seems reasonable that part of the extractable metal pool is derived from the cation exchange complex.

It was further observed than in the more surface polluted Soil I the elements generally had a higher organic-bound fraction.

The HA fraction was separated from the solution at pH 1.5. It is suggested from the literature than few, if any, humic-metal complexes are stable at such low pH. From purely thermodynamic considerations, therefore, most metals bounded to HA in the original pyrophosphate solution should remain in solution after precipitation of HA. However, if the organo-metallic complexes are sufficiently inert, *i.e.* have a slow kinetics of dissociation, the metal may be retained in the HA precipitate. The present results indicate that this may be the case of at least for some metals.

The highest apparent association with the humic fraction is evident for Ca, where typically around 70% of the pyrophosphate extracted amount is found in this fraction. The corresponding percentage for Al, Fe, Cr and Mn is typically of the order of 30%. For the heavy metals Pb, Cu, Zn and Cd the fulvic fraction is even dominant with a few exceptions, but the results show considerable variation. There seems to be no obvious relation between the organic carbon content of the sample and the fulvic-humic ratio for any of the metals.

4. Conclusions

The ratio of organic carbon associated fraction to nitric acid extractable fraction was higher for Cu and Pb than for the other elements, which may at least in part be explained by stronger complexes formed between these metals and humic matter.

The association with the humic fraction is highest for Ca (around 70%) while for Al, Fe, Cr and Mn is typically of order (30%).

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HEAVY METAL DYNAMICS IN SPRUCE (PICEA ABIES) NEEDLE LITTER DURING DECOMPOSITION

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1. Introduction

Litterfall constitutes the major influx of elements to the soil in forest ecosystems. While the nutrient dynamics in decomposing needle litter have been followed in several studies (see e.g. LASKOWSKI et al., 1995), few studies longer than three years have been carried out on heavy metal dynamics in litter on low pollution sites. In a cold climate where decomposition rate is slow, long experimental series are needed to obtain statistically significant long-term trends in heavy metal dynamics during decomposition.

In the study presented here, the dynamics of heavy metals in spruce needle litter were followed at five sites in Southern Sweden for 7 years. The main objectives were 1) to study the change in amount and concentration of Cd, Zn, Cu, Pb and Mn during decomposition and 2) to compare heavy metal dynamics between sites with different climate.

2. Materials and Methods

Incubation of litterbags was carried out for 7 years in five 50-60 years old monocultural stands of Norway Spruce (*Picea abies* (L) Karst) in relatively unpolluted areas in the south of Sweden. The sites are situated between the latitudes 56- 58°N. Mean annual precipitation varies between 530 and 870 mm and mean annual temperature ranges between 5.4 and 6.2 °C (ALEXANDERSSON et al. 1991). The soil consists of sandy till deposits at all sites except one, where glacifluvial sand sediments dominates. All profiles are podzols and the humus is mainly of mor type.

Locally produced brown needle litter was air dried, and incubated in litterbags on top of the litter layer in each stand. During the first two years, the litterbags were collected twice a year and thereafter once a year. The needles were separated from ingrown plant roots *etc* and analysed for mass loss. The samples were digested in 5.0 ml conc. HNO_3, for 25 minutes at 175°C in a tight PTFE bomb in a microwave oven. Digests were analysed for Cu, Zn and Mn with ICP and for Pb and Cu with AAS.

3. Results and Discussion

The amounts of Cd, Zn, Cu, Pb and Mn in the remaining needle litter at all sampling dates and on all sites were subject tò principal component analysis (PCA). PC1 explained 45.8 % of the variance in the data set and PC2 27.5%. Pb and Cu were grouped and had a higher influence on PC1 than on PC2, which also was true for Mn. Positions along PC2 were mainly influenced by Cd and Zn. The clustering of the elements was in accordance with previous studies of their general reactions in soil (see review by BERGKVIST et al., 1989). Furthermore the PCA showed that adjacent sites, with similar climatic conditions had similar heavy metal dynamics.

Mn decreased most in total amount (Table1). Generally, the total amounts of Cd and Zn decreased as well. Pb and Cu increased most in total amounts. However, after about 60% total mass loss, the Pb and Cu amounts started to decrease. Generally, after 7 years the concentrations of Cd, Zn, Cu and Pb had increased at all sites while the concentrations of Mn had decreased

(Table 2). The increase in concentration of an element in the litter can be the result of 1) a lower release of element than litter mass loss, 2) aerial deposition, 3) upward transport by soil organisms, including roots and 4) leaching from overlaying litter. If the input of an element to the litter exceeds the losses, the total amount of the element will increase too.

Table 1. Initial amounts of Cd, Zn, Cu, Pb ($\mu g\ g^{-1}$) and Mn ($mg\ g^{-1}$) and their relative amounts (%) after seven years of incubation.

Site	Cd_{init}	Cd_{7y}	Zn_{init}	Zn_{7y}	Cu_{init}	Cu_{7y}	Pb_{init}	Pb_{7y}	Mn_{init}	Mn_{7y}
R	0.1	86	45	76	2.0	55	1.6	333	2.20	57
Tv	0.1	57	17	168	2.0	77	0.1	6677	1.10	16
To	0.2	55	42	59	2.0	157	5.3	474	0.88	11
M	0.1	62	46	31	2.0	131	4.6	411	1.60	4.3
F	0.1	102	56	33	2.0	93	3.8	399	1.10	5.1

Table 2. Concentrations of Cd, Zn, Cu, Pb ($\mu g\ g^{-1}$) and Mn ($mg\ g^{-1}$) after seven years (7y) of incubation.

Site	Cd_{7y}	Zn_{7y}	Cu_{7y}	Pb_{7y}	Mn_{7y}
R	0.5	200	6.4	31.0	7.30
Tv	0.2	94	5.1	22.0	0.72
To	0.3	68	8.6	69.0	0.26
M	0.2	46	8.4	61.0	0.22
F	0.4	71	7.3	59.0	0.22

According a review by TYLER (1992) there is a risk for reduced microbial activity in the mor layer at concentrations over 3-4 μg Cd g^{-1} dw, 300 μg Zn g^{-1} dw, 20 μg Cu g^{-1} dw or 150 μg Pb g^{-1} dw. As our values are lower (Table 2) and since our concentrations became more constant during the later part of the incubation or even started to decrease (relative the highest measured concentration) at some of the sites, it is unlikely that concentrations negatively affecting the overall microbial activity in the mor layer would be reached during prevailing conditions at these low-polluted sites.

4. Conclusions

1) The overall dynamics of Cd and Zn and to some extent Mn as well as those of Cu and Pb were similar. 2) Although all elements but Mn increased in concentration, levels toxic to microbes were not reached. 3) Geographical location of the sites had a high influence on the heavy metal dynamics during decomposition.

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EFFECT OF ORGANIC WASTES ON SOIL METALS: SIMULATION BY USING HOMOGENEOUS SOIL COLUMNS

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1. Introduction

It is known since early in the 19th century that the presence of organic matter has a strong effect on metal solubility in soils (FÖRSTNER and WITTMANN, 1983). Organic substances can influence metal solubility in several ways, *e.g.* increasing metal solubility by complexation, altering the distribution between oxidised and reduced forms, or influencing the extent to which metals are adsorbed on solid matter (SINGER, 1977). In this work, the influence of various composts on the metal solubility in a soil was studied.

2. Materials and Methods

The power of aqueous extracts of composts of urban solid residues (USR), olive oil industry (OI) or paper industry (WPI) to mobilise soil metals was tested by obtaining breakthrough curves (BTC) of the extracts through homogeneous columns of a sandy soil. The original soil and samples which had received solid USR or OI in the field were studied. The mobilising power of the extracts was compared with that of a simple electrolyte, $Ca(NO_3)_2$. Sequential extraction of the soil metals was also carried out by the BCR protocol (URE *et al.*, 1993).

Table 1: Metal contents in the soil samples and in the aqueous extracts.

Material	Unit	Cu	Zn	Ni	Pb	Mn	Fe
Blank soil	mg kg^{-1}	4.1	23.2	11.4	13.9	109	6090
OI soil	"	10.2	32.9	12.7	17.1	122	6390
USR soil	"	6.0	23.2	11.9	15.5	104	5550
OI extract	mg l^{-1}	0.03	0.27	0.00	0.10	1.09	65.7
USR extract	"	1.44	0.54	0.18	0.09	0.09	2.73
WPI extract	"	1.10	0.18	0.02	0.01	0.28	0.08

3. Results and Discussion

Sequential extraction of the soil metals showed a general increase in the more mobile fractions in the soil samples treated with composts, but for most metals this mobilising action could be explained by the contribution of the metals originally present in the composts. These results are shown elsewhere (DÍAZ-BARRIENTOS *et al.*, 1999).

The BTCs of the untreated (blank) soil showed higher concentrations in the leachate than the treated soils for many metals, especially Fe, Mn, Zn and Pb. The Cu BTCs for the USR extract and those for the OI extract approach the original Cu concentration of the extract for large pore volumes. Mn and Zn are released from the three soils by all the extracts, and a maximum observed in the the first few pore volumes of the BTCs with OI extract is clearly broadened and retarded for the soils amended with composts. Pb and Ni give low concentrations, but significantly higher for the USR extract. The soil amended with USR gives lower Pb concentrations than the other two samples. Leaching with $Ca(NO_3)_2$ gave always very low metal concentrations.

Table 2 shows the net extracted amounts, as percentage of the soil total contents, after subtracting the concentrations initially present in each solution (averages of 2 replicates). The Cu initially present in the USR and WPI extracts was partially retained by the three soil samples, with increases in their Cu contents ranging between 17 and 71 %. On the contrary, the OI extract released some Cu from the three soils. Mn and Zn are released from the three soils by all the extracts, especially by OI, 24 to 34 % and 26 to 57 % of the total Mn and Zn contents.

Table 2: Net metal released from the soil columns, % soil content

		Leaching solution			
Soil	Metal	$Ca(NO_3)_2$	USR extr.	WPI extr.	OI extr.
Blank	Cu	1.51	-36.1	-71.0	3.51
	Fe	0.01	-0.05	0.01	-2.61
	Mn	3.84	5.61	3.93	34.2
	Zn	2.92	10.5	1.60	56.7
	Pb	5.29	-3.55	0.62	5.52
	Ni	1.35	0.86	-0.30	1.94
OI	Cu	0.83	-17.1	-68.2	0.34
	Fe	0.01	0	0.02	-4.18
	Mn	2.93	5.32	5.29	37.8
	Zn	1.10	0.77	2.71	42.1
	Pb	0	5.69	0.29	11.4
	Ni	0	3.61	0	1.04
USR	Cu	1.40	-21.2	-35.2	4.64
	Fe	0.01	-0.06	-0.001	-3.74
	Mn	1.46	2.64	0.76	24.4
	Zn	0.56	4.64	-0.18	26.2
	Pb	1.77	-0.91	0.12	-2.13
	Ni	1.66	-2.96	0	0.02

4. Conclusions

The three extracts show a significant capacity to mobilise some soil metals, especially Mn, Zn, and, in some cases, Pb. On the contrary, Cu originally present in the compost extracts is preferentially retained by the soils. OI extract is the most powerful agent for metal mobilisation. Amendment of the soils with the composts, especially with USR, often lowers the amounts of metals released by the extracts or retards the appearance of the maximum in the BTCs. Therefore, while amendment with these composts may decrease metal mobilisation, their soluble fractions have a mobilising power which can be significant from the point of view of environmental protection.

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C IN THE ORGANIC FRACTIONS AND AVAILABLE OF LEAD IN SOIL CONTAMINATED WITH LEAD AND CULTIVATED WITH BLACK OAT.

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1. Introduction

Soil contamination with heavy metals is today a quite discussed theme, due to the presence of those elements in several materials added to soil. The descontamination processes are not very efficient and, therefore, the use of polluted soils is limited, because the pollutants can be absorbed by plants and enter into the alimentary chain.

In soils of tropical climate, heavy metals can be associated to exchangeable fractions, Fe and Al oxides and organic matter. Organic matter is important in the imobilization of Pb in soil, especially humic acid. Some studies have shown that Pb can interfere in the process of decomposition of organic material present in the soil. Considering such aspects, the present work had for objective to evaluate the effect of rates of Pb in the content of C in the organic fractions in a soil cultivated with oat and the accumulation of the metal in the soil.

2. Materials and Methods

The experiment was conduced under greenhouse condition. The used soil was a Tipic Eutrorthox, medium texture, collected in the layer 0-20cm and presenting the following characteristics: pH $CaCl_2$ = 5.6; OM = 21g dm^{-3}; P = 51mg dm^{-3}; K = 2.7; Ca = 30; Mg = 13; H+Al = 22; S = 45.7 and T = 67.7 ($mmol_c$ dm^{-3}); V = 68%; 340 g kg^{-1} of clay; 20 g kg^{-1} of silt; 330 g kg^{-1} of fine sand and 310 g kg^{-1} of thick sand.

The used plant was black oat (*Avena* spp). The used experimental design was completely randomized, with 4 treatments (0, 200, 400 and 600 mg Pb kg^{-1} soil as Pb Cl_2) and 4 replications. Each pot received 8 kg soil. The pots received water periodically in order to maintain the WHC, and after 44 days (incubation period) 15 seeds of black oat were sowed, being kept 4 plants by pot, when they presented 5 cm of height. All the treatments received a plantation fertilization with: 5mL solution Fe.EDTA, 10g of triple superfosfate and 15 mL of 1mol L^{-1} potassium chloride. Two covering fertilization with nitrogen and potassium were accomplished every 15 days after the sowing by adding 150mg kg^{-1} of N(ammonium nitrate) and 100mg kg^{-1} of K(KCl). The soil samples, obtained at 60 days after sowing, were air dried, sieved to 2mm and analyzed with respect the content of carbon in the organic fractions and available lead (Melich-1).

3. Results and Discussion

In Table 1, the medium values obtained for carbon in the soil organic fractions at 60 days after the sowing of black oat are presented. Total carbon, C in the humin and C in the humic matter did not differ estatisticly in the applied treatments, but the rates of lead affected the content of C in the fraction humic acid. In the treatment 400 mg Pb kg^{-1}soil it was found smaller content of carbon, differing from the other rates of Pb, but not from the control.

Table 1 - Contents of carbon in the organic fractions in soil contamined with lead and cultivated with black oat.

Treatments mg Pb kg^{-1}soil	TC	HMC	HAC	HC[1]
	g kg^{-1}soil			
0	9.653 A	3.403 A	1.415 AB	5.064 A[2]
200	9.553 A	3.434 A	1.155 BC	4.964 A
400	10.150 A	3.652 A	1.015 C	5.483 A
600	9.902 A	3.122 A	1.592 A	5.781 A

[1]CT - Total carbon; HMC - organic carbon in the fraction humic matter; HAC - organic carbon in the fraction humic acid; HC - organic carbon in the fraction humin.
[2]means followed by the same letter did not differ to each other by the Test of Tukey at 5%.

In relation to extracted Pb, there was significant difference among the treatments, and in the rates 400 and 600 mg Pb kg^{-1} soil it was observed larger contents of the metal in relation to the other treatments (Figure 1). The contents of available Pb in the soil could be represented by linear equation: $y = 0,8300 + 0,0534x$ $r = 0,71^{**}$, where y is the content of Pb (Melich-1) and x is the rate of Pb.

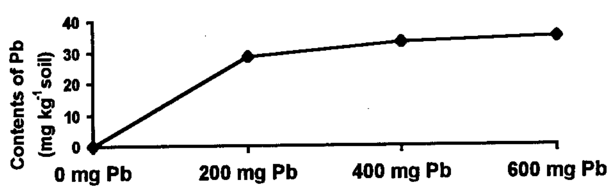

Figure 1 - Contents of extractable Pb of the soil contaminated with Pb and cultivated with black oat, using the extractor Melich-1.

The amount of extracted soil lead was low, showing that a big part of the added Pb should have been complexed with the organic matter, clay or oxides, changing the soluble form of $PbCl_2$ in a no available form to plant uptake.

BINDING STRENGTH AND MECHANISM OF CHROMIUM ADSORPTION ONTO PEAT

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1. Introduction

Besides vast array of applications as a natural sorbent, peat as an almost pure humic-rich organic matter gives a unique opportunity of studying sorption mechanism onto organic matters that is still not clear. The presented study is focused on the elucidation of binding/release mechanism of Cr^{3+}ion onto peat and a role of humic substances in its binding, along with the identification of structural changes connected with metal sorption.

2. Materials and Methods

Studies on sorption/desorption properties of selected peat samples for Cr^{3+} comprised batch and column experiments on metal binding onto homogenized material from synthetic solution in monometallic and binary system (Cr-Cu, Cr-Cd) at pH 4.0, and from real polymetallic waste at pH 1.24, as well as sequential fractionation of bound metals with respect to binding strength and decreasing vulnerability to remobilization (KERSTEN and FORSTNER, 1988). Six consecutive fractions reflecting equivalently increasing binding strength were identified, from F0(PS) to F5(OM). From the selected peats, humic and fulvic acids (HA and FA) were extractrd with use of a method adapted from the IHSS extraction procedure. Dissolved fraction has been flocculated with Cr^{3+}under batch conditions and characterized by instumental analytical methods (elemental analysis, UV-VIS, FTIR, TOC, NMR) and in particular by high performance capillary electrophoresis (HPCE), in order to identify structural changes connected with metal sorption and mechanism of metal binding.

3. Results and Discussion

Sorption capacity of peat for Cr^3 appeared to be particularly high, strong binding in the "insoluble organic" F5(OM) fraction being the prevailing form, specific for this metal. In batch system, moderately reducible F4(MRO) phase attributed to the formation of HA chelating complexes was also of a considerable significance (15-28 %). Minor amounts of bound Cr^{3+} were distributed in the labile fractions, following the order F5(OM) > F4(MRO) > F1(EXC) >> F2(CARB) > F3(ERO) >> F0(PS). The deep acidification of equilibrated solution to pH 2.2-3.7 indicated extensive hydrogen displacing by Cr^{3+} ions from functional groups of humic substances. Sorbed Cr^{3+} shows very low susceptibility to release (6.1-6.4 %.) Metal binding onto peat matrices in binary systems (Cr-Cu) and (Cr-Cd) showed strong competition between these ions (Fig.1A), with profound domination of Cr^{3+}over these metals for the sorption sites, and particularly high suppression of Cd^{2+}. The low increase of a total sorption capacity for both ions in the binary system compared to the monometallic system proves a limited spare capacity in the sites jointly occupied by both metals. Increased acidification of the equilibrated solution (up to pH 2) showed that only strongly bound fraction of $Cu^{2+}$and Cd^{2+}could successfully compete for sorption sites with Cr^{3+}.

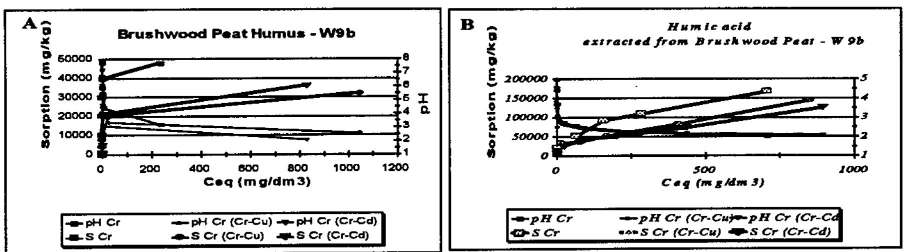

Figure 1: Equilibrium mass isotherms for Cr^{3+} onto peat (A) and HA extracted from peat (B), and pH of equilibrated solutions in monometallic (Cr only) and binary systems (Cr-Cu and Cr-Cd) at S/L=1:10. Input solution: pH 4.0, c_0= 1 – 5000 mgMe dm^{-3}

Concurrent batch sorption studies carried out on the solubilized humic acids (HA) extracted from peat proved these compounds, which belong to "soluble organic" fraction, to be good adsorbents for Cr^{3+}. The FTIR spectra of studied peats and extracted HA show lower amounts of aliphatic (from waxes) and carbohydrates (free sugars, cellulose), and higher phenolic compounds.. From CZE analysis, the phenolic equivalents for HA extracted from peat were estimated for 12-15 %. Changes in charge densities during the flocculation followed by CZE and NMR spectroscopy showed a rather aliphatic and carboxylic character of humic fractions strongly reacting with Cr^{3+} Strong acidification of the equilibrated solution indicates extensive displacement of H_3O^- by Cr^{3+} ions from carboxylic sites. Effect of divalent metal ions (Cu^{2+}, Cd^{2+}) on Cr^{3+} for sorption sites onto HA in the binary batch system appeared to be stronger and considerably different from that for the peat matter (Fig 1 B). The sorption capacity of HA for Cr^{3+}decreased in the presence of both ions. The suppressing effect of Cd^{2+} was found to be particularly strong (25-37 %) and was not accompanied by parallel binding of this metal on HA. This means, that Cd^{2+}has, entirely screening and not competing effect. Partially, the similar screening effect showed also Cu^{2+}, simultaneously with competing sorption.

Dynamic sorption at the same parameters of the input solution displays higher efficiency than the batch process. Cr^{3+} ions appeared to be bound stronger, mainly due to further Cr^{3+}enrichment in the "insoluble organic" F5(OM) fraction parallel to its depletion in "soluble organic" fractions. In dynamic process and in the systems with critical parameters (low input pH, high other metal ions competition) the role of the "insoluble organic" fraction F5(OM) appears to be particularly important.Cr^{3+}affinity to this fraction become predominant, mainly as a result of the transformation of a primary fractional structure.

4. Conclusions

The fraction structure of Cr^{3+}enrichment displays the predominant strong binding in the "insoluble organic" fraction of organogenic matter, which consists of humins, lignin and cellulose. The role of "soluble organic" substances in Cr^{3+} sorption decreases in time and under long-term dynamic conditions or at critical parameters (low pH, strong competition) becomes marginal.

HUMIC ACID FROM ENDEMIC AREAS OF ARSENICOSIS IN INNER MONGOLIA AND TAIWAN—A COMPARATIVE STUDY

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1. Introduction

Endemic arsenicosis was discovered in the early 1990's in the Hetao plain, the central and western Inner Mongolia of China(Luo Z.,1993). In local underground water was found a dual high level of both As and HA(humic acid),as is observed in the Blackfoot disease area in Taiwan. Blackfoot disease is characterized by thrombo-embolism, especially in lower legs. Some authors consider that Blackfoot disease is caused by the high As content in drinking water, but the symptoms of Blackfoot disease are quite different from those of typical arsenicosis. A unique fluorescent substance was found in drinking water from this area in 1975 by Lu F.J. and it has been turned out to be HA by later studies(Lu F.J., 1990). In order to shed light on the pathogenesis of Blackfoot disease with the concern as to whether the disease may also occur someday in arsenicosis affected areas in Inner Mongolia, the physical and chemical characters of HA extracted from drinking waters from the above two regions were comparatively studied.

2. Materials and Methods

The As concentrations and physical and chemical characters of water samples and of HA samples from the above two regions were measured by using AAS, ICP-MS, IRS, FS, TLS(total luminescence spectroscopy),etc. Ames test(Ames,1975) and lipid peroxidation experiments(Steven ,1987) were also conducted. The water samples of Inner Mongolia used in this paper were collected from 51 farmer families in the Alxa League, the Bayannur League and the suburbs of Hohhot City , and the water samples of Taiwan were from 1189 drinking water wells distributed in 38 villages in Yunlin, Jiayi, Tainan and Pingdong counties(Lu F.J.et al.,1989).

3. Results and Discussion

The analytical results show that the water samples from Inner Mongolia were relatively alkaline (pH=7.91 on average). The average concentrations of cations vary in the order of $Na^+ > Ca^{2+} > Mg^{2+} > K^+$ while those of anions follow the decreasing order of $HCO_3^- > Cl^- > SO_2^{2-} > CO_3^{2-}$. The underground waters are assigned to the HCO_3^-–Na and HCO_3^-–Cl^-–Na types. The water samples are high both in fluorescence intensity and in COD and contain a lot of HA with the same fluorescence characteristics as those observed in the Blackfoot diseased areas of Taiwan.
The sampling sites are located in the piedmont alluvial-diluvial slope plain between the Mt. Langshan and the Mt. Daqingshan and the alluvial-lacustrine plain between the Yellow River and the Dahei River in Inner Mongolia. The underground water contains abundant HS ,CH_4 and HA(Yu X.et al,1996). That is because in the low land a lot of oxygen had been consumed in the process of oxidation of organic matter, thus resulting in a highly reducing environment. Under such condition, it is found that the As concentration(0.53 mg L^{-1} on average) of water samples is more than 10 times over the drinking water standard of 0.05mg L^{-1} specified by WHO as well as

the Chinese Government. In the reducing environment As would occur mainly as As^{3+}. Comparisons are made between the water samples from two regions, they are similar in pH, fluorescence intensity and TDS (total dissolved solids), except for the apparently higher As concentration for the Inner Mongolia samples, and both samples are considerably high in As with strong fluorescence, and have apparently positive correlations among As contents, COD, fluorescence intensity, pH and TDS.

The water samples were further studied using fluorescence spectrometry, IRS and TLS.As the results show that samples from the two regions are similar in fluorescence spectra but differ somewhat in IRS and TLS, the difference may be a reflection of the differences in radicals and structure of HA owing to different hydrogeological conditions in the two regions, and also be related to the difference in biological effects, i.e., in-vitro experiments led us to find that HA can cause lipid peroxidation of unsaturated fatty acid sodium salts, though such the tendency is relatively weak and unstable, It is observed that 0.05mM Fe^{2+} can promote this reaction,and HA from Inner Mongolia has a stronger ability to cause lipid peroxidation than that in samples from Taiwan. While HA from Taiwan exhibits a more prominent effect of mutation with respect to TA98(⊕S9) but HA from Inner Mongolia is of no such effect.

4. Conclusions

Well water samples from the two regions share much in common with respect to their physical and chemical properties. However, they are quite different in As content and in the structure of HA. This difference decides that they are quite different in biological effects. For this reason, it is considered that Blackfoot disease is unlikely to be expected in the endemic areas of arsenicosis in Inner Mongolia, at least in the near future.

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Analytical Tools
(Technical Session 1)

USE OF CHEMICAL AND PHYSICAL FRACTIONATION TO ASSESS CADMIUM SPECIATION IN SOME SOILS OF THE SWISS JURA

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1. Introduction

Recent studies have pointed out that large Cd concentrations (up to 15 mg/kg) could be measured in some soil of the Swiss Jura, even their have not been polluted by human activities (Liebig and Dubois (1997)., Dubois et al.(1998). Theses Cd concentrations exceeded the guide value, fixed at 0.8 mg/kg, by the Swiss legislation. Therefore some measurements of the chemical behaviour and mobility of heavy metals are required in order to understand the availability and the fate of heavy metals in soils and to predict the environmental risk. The main objective of this paper is to assess the Cd distribution in the soil compartment using the chemical and physical fractionation.

2. Materials and Methods

The chemical and physical speciation were carried out in eight soil profiles. These profiles were representative of the main soil types of the Swiss Jura. A new scheme of sequential extraction, especially adapted for these samples (Benietz & Dubois 1998) was applied. This scheme consisted of six steps expected to extract: exchangeable Cd (1) and Cd bound to carbonate (2), Mn-oxides and amorphous compounds (3), Fe-oxides (4), organic matter (5) and residual fractions (6). On the other hand, the Cd content of six particles sizes (> 200, 200-100, 100-50, 50-20, 20-2 and < 2 µm) was measured in order to study the relationship between Cd distribution and particles size in the soil. The total Cd content in the samples was analysed after digestion with acid mixture under pressure using a microwave oven. The Cd was measured by electrothermal atomic absorption spectrometry (GFAAS) and the other elements by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP).

3. Results and Discussion

The main types of soils were Leptosols and Luvisols (FAO-UNESCO 1988). They lie on hard calcareous rocks of the Malm stage(Kimmeridgian or Sequanian) and on Marl or limestone of the Dogger stage (Bajocian, Bathonien), but they were all decarbonated in the upper layer. The Cd distribution in the soil profiles showed that Cd was accumulated in the first horizon and in the horizon directly in contact with the bedrocks. The Cd enrichment of the deepest horizon was due to rock weathering but the reason of Cd increase in the upper horizon is not clear. Indeed, such an Cd accumulation, commonly attributed to antropogenic inputs could be discounted in our case (Dubois et al., 1998). The sequential extraction results (figure 1) showed that Cd was rather bound to organic matter in the upper horizons and to Mn-oxides and amorphous compounds in the deeper horizons. The physical speciation results (figure 2) showed that the largest Cd concentrations were found in the smallest size particles whatever the horizon as it has been reported by Lee et al.(1997) and Ducaroir et al.(1995). Nevertheless, the Cd concentration of the coarse fraction is not negligible and furthermore increased from the bottom to the top of the profile. The physical speciation showed also that the coarse fractions was mainly composed by vegetal residues and therefore the results suggested that a large part of Cd was uptaken by plants and accumulated in the humus layer after their decay. This fact might explain the accumulation

of Cd observed in the upper horizons. Further studies are in progress to confirm the role of plants in Cd turnover.

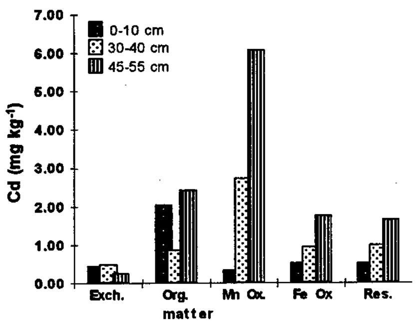

Fig 1. Chemical speciation of profile 44P1

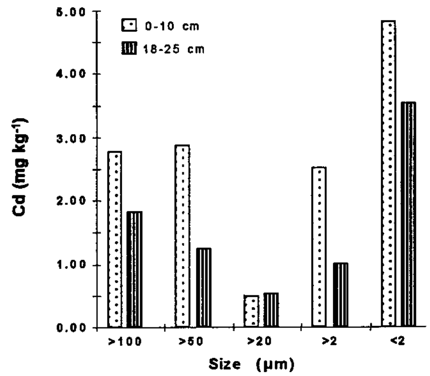

Fig 2. Physical speciation of profile MA2

4. Conclusions

The chemical and physical speciations are useful methods to assess Cd distribution in the soil profiles of the Swiss Jura. Our results showed also that Cd is preferentially bound to organic matter in the upper horizons whereas Cd was bound to Mn-oxides and amorphous compounds in the deeper horizons. Moreover, although a largest part of the Cd content was accumulated in the clay fraction, the Cd content in the coarse fractions could not be discounted, especially in the upper horizons. These horizons contain some vegetal residues which are the main source of Cd.

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MEASURING AGEING OF METAL CONTAMINANTS IN SOIL USING ISOTOPIC TRACERS AND ΔL VALUES

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1. Introduction

It has been known for some time that after addition to soil of soluble metal, availability of the metal decreases with time. Initially this decrease is associated with adsorption of metals to soil surfaces, but longer-term, slower "fixation" reactions appear to proceed, following adsorption, which continue to reduce metal bio- and phytoavailability. This study proposes a technique to quantify these slow reactions using isotopic tracers.

Theoretical Basis After addition of soluble metal radioisotopic tracer to soil (R_{soil}), the isotope exchanges with metal ions in soluble forms in soil solution and with ions on the exchange complex. After a period of time, plants are grown and metal concentrations (M_{pl}) and radiotracer activites (R_{pl}) in the plant determined to calculate the amount of plant available metal in soil, the "L" value (Larsen, 1952) is determined as;

$$L = \frac{M_{pl}}{R_{pl}} \times R_{soil} \qquad [1]$$

It is hypothesised that L values are not constant but change with time of contact of isotope with soil (experimentally excluding effects due to rhizosphere processes which mobilise metals), and that this rate of change in L value (ΔL value) can be used as a measure of the ageing reactions which metals undergo in soil. By measuring L values after different periods of isotope:soil contact (t), L values at a given time can be expressed;

$$L_t = L_0 \exp(-kt). \qquad [2]$$

where k is an "ageing" rate constant, or

$L_t = L_0 n^t$

where $n = \exp(-k)$. By comparing L values at two times separated by u days, ΔL values are

$$\Delta L = L_t - L_{t+u}. \qquad [3]$$

Then $\Delta L = L_0 \exp(-kt) - L_0 \exp(-k(t+u)) = L_t(1-\exp(-ku))$. Re-arranging we have

$$1 - \frac{\Delta L}{L_t} = \exp(-ku), \text{ or} \qquad [4]$$

$$k = -\ln(1 - \frac{\Delta L}{L_t})/u = -\ln\left(\frac{L_{t+u}}{L_t}\right)/u. \qquad [5]$$

2. Materials and Methods

Two soils were incubated moist at 20°C with carrier-free ^{109}Cd, ^{63}Ni and ^{65}Zn for periods of 52 and 404 days, and a further treatment was incubated at 40°C for 404 days. Subsequently, wheat (*Triticum aestivum*) plants were grown on the soils for 22 days, and specific activities of isotopes in the plant material and soil determined. In order to compare results between soils, L values were expressed as a fraction of total metal in soil.

Table 1. Selected soil characteristics.

Characteristic	pHw	Total Cd ($mg\ kg^{-1}$)	CEC ($cmol^{+}\ kg^{-1}$)	Oxalate-extractable Al ($mg\ kg^{-1}$)	Fe	Dominant mineral
Soil 1	8.5	0.139	13.6	670	400	Illite
Soil 2	5.5	0.265	15.0	17,	11,4	Allophane

3. Results and Discussion

Data for Cd only are presented. The fraction of total Cd which was labile was significantly ($P<0.001$) different between the soils. In Soil 1 there was no effect of time on Cd lability, while labile Cd in Soil 2 decreased (Figure 1) with time ($P<0.05$). Temperature had no significant effect ($P<0.05$) on L values, although data suggested an increasing trend with higher temperature of incubation.

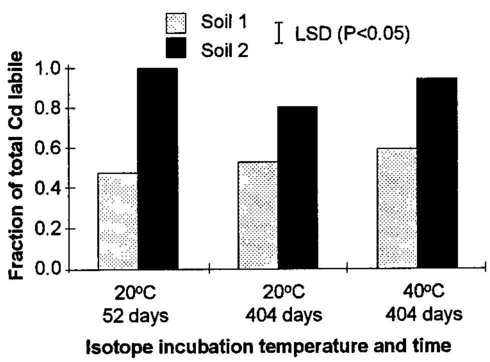

Figure 1. Labile Cd in relation to time and temperature of incubation.

This latter result contrasts with the conclusions of Barrow (1997) who suggested time and temperature have consistent effects on metal availability as determined by batch sorption experiments. The rate constant (20°C) for Cd ageing in Soil 2 was 0.23 y^{-1} and in Soil 1 was not significantly different from zero. The results can also be compared to those of Hamon et al. (1998) who showed, using a related L value technique, Cd fixation rates of approximately 0.01 y^{-1} in an acidic pasture soil.

4. Conclusions

These data indicate the need to determine the ageing rate constants in a wide variety of soils to determine which soil characteristics control metal ageing reactions.

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LEAD ISOTOPE RATIO DETERMINATIONS IN SOIL SOLUTION SAMPLES BY MEANS OF ICP-SMS

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1. Introduction

Although the potential of inductively coupled plasma mass spectrometry (ICPMS) for isotope ratio measurements has been recognized from the early days (Mook etal., 1981) up to now most instruments have been used for element determination only. This is mainly due to the relatively poor isotope ratio precision of ICPMS in comparison with more "traditional" isotope ratio techniques such as, e.g., thermal ionization mass spectrometry (TIMS) and gas source mass spectrometry (GSMS). However, in spite of the relatively limited isotope ratio precision attainable, ICPMS has been the method of choice for some applications as a result of its ease of operation, high sample throughput, and widespread availability. Hence, in the literature, isotope ratio measurements have been reported for among other archaeological, geological , environmental, and nutritional studies and for obtaining a more profound insight into chemical reactions (e.g. Latkoczy etal., 1998) While the use of ICPMS for the determination of isotope ratios for a number of elements in clinical, geological, and environmental samples has been described, by far the most commonly studied element is lead (Date etal., 1987) The main source of lead in the atmosphere is the emission from gasoline combustion. This lead displays the isotope composition of ores from it which it was derived before its addition to gasoline. After the emission of anthropogenic and geogenic derived lead into the atmosphere, polluted air masses are transported from the source by strong convective mixing processes and are finally deposited in the soil during precipitation events. Therefore, measurement of lead deposition examined through depth profiles in soil samples can be used to track anthropogenic sources of lead. Similar investigations have been done in snow and ice of high alpine glaciers, similar to polar ice sheets, to determine trends of atmospheric air pollution. This presentation explores the suitability and application range of a commercially available Inductively Coupled Plasma Sectorfield Mass Spectrometer (ICP-SMS) (Finnigan MAT Element) in determining quickly, precise and accurate stable isotope ratios within various samples for different applications in soil sciences.

2. Materials and Methods

To differentiate between anthropogenic and geogenic sources of lead in soils and its potential danger due to its mobility in soil and hereafter to the food pathway, lead concentrations and the isotopic composition were determined by ICP-SMS in soil solutions collected from a site in Burgenland, Austria. All experiments were performed by using the Finnigan MAT Element ICP-mass spectrometer (Finnigan MAT, Bremen, Germany). Scan conditions were adjusted in order to obtain optimum precision and accuracy. Instrumental parameters were optimized for isotopic ratio determinations including sampling time, settling time of the magnet, segment duration, number of samples, mass window, and total scan duration to achieve accurate data. The experimental relative standard deviations were calculated on the basis of five successive measurements each. All chemical preparations were conducted on special class 100 work benches inside the class 10.000 clean lab. A stock solution of 1.000µg/L total lead concentration

was gravimetrically prepared using the NIST SRM 981 Lead (Isotopic, high purity metal) with a certified isotopic composition. Thereof, calibration standards were diluted to various concentrations containing 1% (v/v) nitric acid. These working solutions were stored in polyethylene bottles. The soil samples were digested by means of microwave digestion using high purity nitric acid, hydrochloric acid, hydrofluoric acid, and boric acid.

3. Results and Discussion

Measurements of lead isotope ratios and lead concentration on digested soil samples along the whole soil profile have been performed by means of the optimized system parameters described above. All values obtained have been corrected regarding both to deadtime and to mass bias in reference to the certified standard NIST SRM 981. The samples differ significantly in their isotopic composition.

4. Conclusions

From the analytical investigations it can be concluded that there is an anthropogenic impact into the soils (see Figure 1). Further investigations will be performed in combination with a sequential extraction scheme (Zeien and Brückner, 1989) to clarify the mobility of lead.

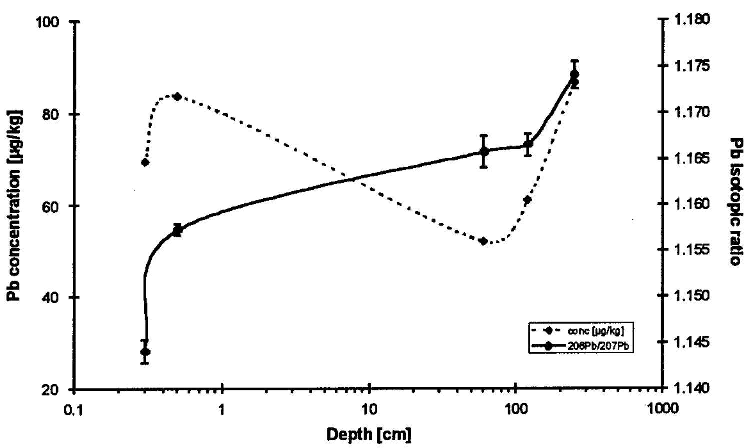

Figure 1: Lead concentration and isotopic composition along the soil profile

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SUBSTRATE- AND ELEMENTSPECIFIC RELATION OF REAL TOTAL VERSUS AQUA REGIA SOLUBLE CONTENTS OF HEAVY METALS IN SOILS

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1. Introduction

In Germany a soil protection act has been put into force at march 1st, 1999. According to the technical annexes soil pollution shall be evaluated following a three level ranking system with precautionary, trigger and action values. The precautionary levels shall sustain a multifunctional use of soils and therefore are equalized with background values in soils. For this purpose there has been an undiminished effort to harmonize Germany-wide data sets for heavy metals in soils since several years. Referring to pedoregional and land use differentiated representativeness (UTERMANN et al. (1998)) a large amount of soil profiles sampled and analyzed in a standardized way is needed for deriving heavy metal background values in soils. In Germany, heavy metal contents in soils are analyzed mainly as either real total contents (X-ray fluorescence, total acid digestion with HF) or aqua regia soluble contents. One way to enhance the data set available for deriving background values is to transform real total into aqua regia soluble contents (or vice versa) on a statistically reliable basis. Existing studies on the relation between the above mentioned methods for the analysis of heavy metal contents in soils mostly reveal single transformation coefficients and are limited to certain substrates or do not apply explicitly for naturally occurring heavy metal concentrations in soils (SUTTNER (1996), RUPPERT (1991)). This work presents a comprehensive experimental approach to determine the relation of real total versus aqua regia soluble contents of heavy metals in soils. It takes into account a differentiation according to substrate and element specific ranges of concentrations relevant to background values in soils.

2. Materials and Methods

Against this background 1969 soil profiles comprising a total of 17050 soil samples were grouped according to their parent material into 11 groups. From each group up to 50 samples were chosen taking into account the distribution of element concentrations and the spatial distribution of the selected soil profiles. These soil samples were analyzed for real total contents (X-ray fluorescence (Cr) or total acid digestion with HF (Cd, Cu, Ni, Pb, Zn)) and for aqua regia soluble contents. Measurements were performed using Inductively Coupled Plasma Atomic Emisson Spectrometry (ICP-AES) (Zn) and ICP Mass Spectrometry (Cd, Cu, Ni, Pb). The statistical analysis comprises a test on data distribution and linear regression modeling.

3. Results and Discussion

Most of the 66 analyzed data sets (11 substrate groups * 6 elements) reveal lognormal distributions. In these cases statistical analysis have been performed with log-transformed data. For 57 data sets a fairly high goodness of fit with $r^2 \geq 0,7$ (s. **tab. 1**) between the two methods can be stated. Weaker relations are limited to chromium, where aqua regia obviously extract varying proportions of the total element content.

Table 1: Number of samples (n), goodness of fit (r^2) and percentage proportion of aqua regia soluble contents (ar) with regard to real total contents (rt) at the lower and upper application limits of the regression equations

SPM[1]	Cd		Cu		Ni		Pb		Zn		Cr	
	n	r^2	n	r^2	n	r^2	n	r^2	n	r^2	n	r^2
AIMR	25	0,79	25	0,99	25	0,98	25	0,84	45	0,91	49	0,70
BIMR	25	0,94	25	0,92	25	0,97	25	0,97	47	0,89	47	0,83
CST	25	0,96	25	0,95	25	0,97	25	0,98	45	0,87	45	
LOE	25	0,91	25	0,96	25	0,92	24	0,91	49	0,74	50	0,40
LST	25	0,91	25	0,95	25	0,90	22	0,88	42	0,85	48	0,50
MBTS	24	0,78	50	0,98	48	0,81	48	0,95	49	0,98	47	0,50
MST	25	0,93	25	0,96	25	0,94	25	0,94	43	0,81	45	0,66
TILL	26	0,91	26	0,96	26	0,97	26	0,95	51	0,84	48	
SAN	37	0,89	49	0,96	47	0,96	49	0,91	49	0,98	35	0,60
SLOE	36	0,82	41	0,95	42	0,95	43	0,97	46	0,78	48	0,34
SST	25	0,81	25	0,90	25	0,96	25	0,88	48	0,92	49	0,68
	ar/rt [%]		**ar/rt [%]**		**ar/rt [%]**		**ar/rt [%]**		**ar/rt [%]**		**ar/rt [%]**	
AIMR	67 - 78		77 - 114		106 - 90		47 - 113		63 - 85		85 - 66	
BIMR	42 - 78		91 - 96		81 - 93		76 - 106		81 - 82		59 - 53	
CST	47 -110		73 - 119		92 - 96		89 - 103		87 - 87			
LOE	38 - 69		60 - 89		91 - 84		69 - 103		108 - 71			
LST	51 - 82		85 - 95		87 - 95		93 - 91		83 - 95			
MBTS	103 - 114		81 - 104		82 - 86		50 - 86		54 - 107			
MST	56 - 89		98 - 95		91		93 - 84		79 - 77		56 - 55	
TILL	45 - 86		79 - 92		66 - 96		58 - 105		82 - 71			
SAN	47 - 94		58 - 108		141- 97		39 - 111		86 - 97		46 - 31	
SLOE	22 - 74		59 - 104		69 - 95		43 - 134		96 - 88			
SST	42 - 69		58 - 91		73 - 86		57 - 105		61 - 86		35 - 27	

[1] SPM = Soil Parent Material:
AIMR = acid igneous and metamorphic rocks; BIMR = basic igneous and metamorphic rocks; CST = claystones; LOE = loess; LST = limestones; MBTS = marine, brackish, tidal sediments; MST = marlstones; TILL = till; SAN = sands; SLOE = sandloess; SST = sandstones;

The percentage proportions of aqua regia soluble (ar) content with regard to real total (rt) content vary in dependence of both, the element concentrations and the soil parent material. The linear regression equations for the mostly lognormaly distributed data sets exhibit an intercept nonequal to zero and a slope nonequal to one. This causes varying transformation coefficients within the concentration ranges relevant to heavy metal background values.

4. Conclusions

The results of the comparison between different digestion methods for heavy metal contents in soils clearly indicate, that a transformation between real total content and aqua regia soluble fraction i) should be performed on the basis of regression functions instead of single transformation coefficients and ii) should be differentiated element- and substrate-specifically. The validity of the regression functions is strictly limited to a given range of element concentrations.

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HEAVY METAL ANALYSIS IN CERTIFIED ENVIRONMENTAL SAMPLES WITH MICROWAVE DIGESTION AND ICP-AES, ICP-MS AND GF-AAS.

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1. Introduction

Nowadays the use of microwave oven digestion associated with the most advanced spectroscopic techniques as ICP-AES, ICP-MS and GF-AAS has proved to be suitable to obtain high levels of precision and accuracy and good recoveries in the determination of the concentrations of trace elements in environmental samples like soils, rocks, sediments, plants, sludges, fly ashes and so on. The advantages of microwave digestion technique over open traditional systems have been extensively observed by several authors, LAMOTHE et al. (1986).These advantages are related to less risks of external contamination, less time needed and the absence of the contamination resulting from the use of large quantities of acids. Nevertheless a risk of losses of relatively volatile elements also remains. The advantages reached by the use of modern spectroscopic techniques as ICP-AES, ICP-MS have been widely recognized by a large number of published papers, BETTINELLI et al.(1989). With the use of these techniques is possible the simultaneous determination of several trace elements in a short period with high accuracy and precision. Aim of the paper is the optimization of the procedures for the determination of several trace elements (Cd,Co,Cr,Cu,Mn,Ni,Pb,Zn) in variuos environmental samples (soils, sediments, biological materials, sludges) using microwave acid digestion and ICP-AES, ICP-MS and GF-AAS through a ring test carried out in two laboratories. During the digestion a systematic investigation was carried out on the power, the type and volume of the reagents, the duration time, in order to optimize the conditions of analysis of single metals in every environmental matrix. Trace metal soluble in aqua regia were determined in all environmental samples except for sediments where total metal content was also determined using a mixture of HNO_3 - HCl and HF.

2. Materials and Methods

The validation of the developed method was carried out with the use of the following standard reference materials: (a) Community Bureau de Reference (BCR) : Calcareous Loam Soil CRM 141R, Light Sandy Soil CRM 142, Sewage Sludge Amended Soil CRM 143, Estuarine Sediment CRM 277, River Sediment CRM 320, Sewage Sludge CRM 144, Sewage Sludge CRM 145, Lichen (*Pseudovernia Furfurea*) CRM 482, Rye Grass CRM 281; (b) National Institute for Standards and Technology (NIST) : Tomato Leaves SRM 1573, Pine Needles SRM 1575. All these materials were dried according to the instructions of the suppliers. In most cases the concentration of trace elements were obtained from the certificates of analysis, in other ones as "best values" from other sources. Two microwave ovens (Milestone 1200 and CEM 2000 models) were used for the digestion of the samples. In the present study two different acid mixtures were used: (a) 8 ml acqua regia, (b) 7 ml HNO_3 + 3 ml HCl and 2 ml HF; all digestions were carried out using 250 mg samples which were brought to a final volume of 50 ml. The analysis of Cd,Co,Cr,Cu,Mn,Ni,Pb and Zn were carried out in two laboratories with two different ICP-AES instruments (Perkin Elmer Optima 3000 and Jobin Yvon 24) equipped with

cross flow nebulizer. Other trace elements were determined by ICP-MS (Perkin Elmer Elan 5000) or alternatively by GF-AAS (Perkin Elmer Zeeman 5100 spectrometer) techniques.

3. Results and Discussion

In the present work quantitative recoveries (>94%) for Cd,Co,Cr,Cu,Mn,Ni,Pb and Zn were obtained for the biological matrices dissolved by aqua regia in a microwave oven. The precision (expressed as RSD%) was generally better than 5-8% and the reproducibility, obtained analyzing the same samples by ICP-AES in two different laboratories, for many elements ranged from 15 to 20%. Similar results were achieved for Cd,Cu,Cr,Ni and Pb analyzed by GF-AAS while ICP-MS was used for the determination of analytes present at concentrations lower than 0.5 µg/g. The evaluation of the method detection limit (MDL) which represents the realistic performance of the complete method, was done by considering the variability of the analytes in blank tests prepared, in different days, according to the described procedure. The analysis of geological materials, soils and sediments, dissolved by nitric/hydrochloric acid digestion led to good results for some elements Cd,Cu,Ni,Mn and Zn (recoveries ranging between 88 and 103%) with lower results for Cr (70-96%) and Pb (68-90%) determined by ICP-AES. However quantitative recoveries were also obtained for Cr,Mn and other elements following an aqua regia/hydrofluoric acid digestion which proved to be the most effective for the determination of the elements associated with siliceous materials.

4. Conclusions

Biological samples generally consist of a complex mixture of carbohydrates, proteins and lipids; therefore before their analysis it is always necessary a dissolution of the organic matter to allow the sample matrix to release the metals bound to it. The use of strong oxidizing agents as nitric acid is sufficient to decompose the organic matrix of the sample and to release many elements as soluble nitrate salts. Other acids can be employed to break down further the sample, according to the elements to be determined and to the chosen analytical technique. Hydrochloric acid is a good solvent for many metal oxides while the use of hydrofluoric acid is necessary for the determination of a number of elements associated with siliceous materials. The wide range of sample compositions represented by environmental materials, often doesn't permit the use of a unique single digestion procedure. For example, sediment samples are one of the most difficult matrices to be digested since they are a mixture of different materials: clay,organic materials, siliceous minerals and refractory oxides. In some cases, to attain a complete digestion the use of HF is necessary to decompose resistant minerals. The results shown in the present paper suggest that a number of elements as Cd,Cu,Ni,Pb and Zn can be easily released after a digestion with nitric and hydrochloric acid only, while for other analytes, Cr and Mn, associated with more resistant minerals, the additional use of HF may be required. The microwave oven acid digestion has proved to be a very rapid and accurate technique for decomposing several types of environmental matrices. The solution prepared by this procedure is suitable both for ICP AES, ICP MS and GF AAS analysis.

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A SEQUENTIAL CHEMICAL EXTRACTION PROTOCOL FOR THE SIMULTANEOUS EVALUATION OF ARSENIC AND CADMIUM MOBILITY.

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1. Introduction

Arsenic contamination in the environment has arisen in several countries as a result of mining and smelting activities. Large quantities of As have been observed in soils situated around gold mining operations where it is extracted as a by-product (range 120-52600 µg/g of As)[1], while typical As concentration in uncontaminated soils range from 1 to 40 µg/g [2]. Considering that it is the chemical and mineral forms of this metalloid that will influence its bioavailability, it is necessary to determine the arsenic phase associations in solids.

Detailed characterisations of arsenic and other trace element behaviour for environmental studies, are often carried out using sequential schemes [1],[3]. Arsenic is a metalloid element, but is often inaccurately referred to as a metal. The feasibility of using methods designed for transition metals for fractionating soil As has been questioned specially, since As is present as anions in soils. An alternative approach has been to use sequential schemes designed to study the forms of P in soils, since the chemistry of As in soils has apparent similarities with that of P (both commonly form oxyanions in the +V oxidation state as they are located in the same column of the periodic table) [2].

2. Materials and Methods

We compare the results obtained for arsenic and cadmium in contaminated and uncontaminated soils using two different sequential extraction schemes: Woolson [4] and Tessier [5] protocols.The Woolson protocol is designed to study the forms of P in soils considering five fractions: water soluble (DIW); Al associated (NH_4F 1st); Fe and organic-Fe associated (NaOH), Ca-associated (H_2SO_4), occluded Al- associated (NH_4F 2nd) and residual. The Tessier protocol considers five geochemical phases: Exchangeable, carbonated, reducible, oxidizable and residual. The studied soils originate from: atmospheric influenced area close to As mining (1), As mine waste (2), industrial site (3), and a reference material (4) ranging from 46 to 135000 µg/g for As and ranging from 0.19 to 1.74 µg/g for Cd. The As and Cd concentrations in the extracts were analysed by ICP-MS.

3. Results and Discussion

Figure 1 shows the results obtained for As by Woolson and Tessier methods for As. Using the Tessier results we can consider As as not potentially bioavailable. In contrast Woolson method shows a large percent of As solubilisable by sodium hydroxide, therefore associated to iron and organic-iron soil components. The reference material results shows that the Tessier method was

not able to solubilise all of the As content in the sample (68 % recovery) , while better recovery was obtained by the Woolsson method (115%).

The results for Cd (figure 2) show that the Tessier method can give more detailed information about its association with specific solid phases. The Woolson method for Cd shows that only sulfuric acid is able to solubilise Cd, but this strong acid can destroy not only carbonates but also other mineralogical phases, generaly solubilized in the residual fraction of Tessier method. Better recovery was obtained by the Tessier method for Cd in the reference material (113%) in contrast with the Woolson method (60%).

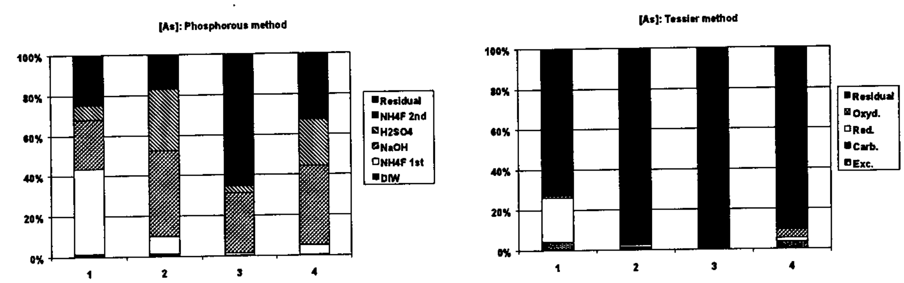

Figure 1: **Sequential Extractions Methods for As.**

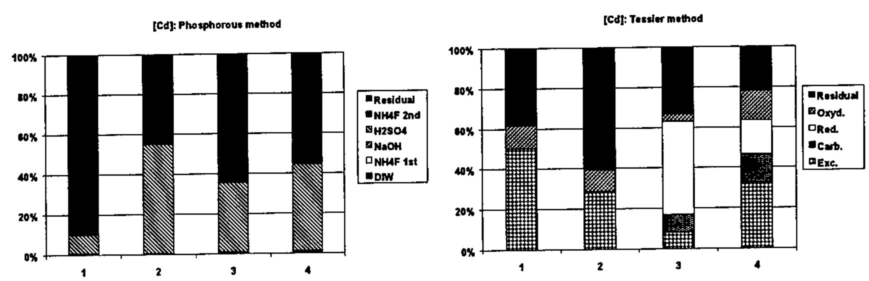

Figure 2: Sequential Extractions Methods for Cd.

4.. Conclusions

Evaluating these results we propose a single protocol to assess simultaneously anion and cation speciation in solids as follow: *Carbonated Phase*: CH_3COONa (0.6 M) at pH 5 ; *Reducible Phase* : $NH_2OH.HCl$ (0.1 M) & HOAc 25 % (v/v) ; *Oxidizable Phase:* H_2O_2 (30 %) & HNO_3 (0.02 M) (5:3); *Alkaline Phase:* NaOH (0.1 M); *Residual Phase:* HNO_3 / HCl (1:3) & HF.

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Trace Elements in Agricultural Ecosystems

(Technical Session 2)

ATMOSPHERIC DEPOSITION OF HEAVY METALS ONTO AGRICULTURAL LAND IN ENGLAND AND WALES.

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1. Introduction

Atmospheric deposition is the major source of heavy metal inputs to agricultural land except where metal-rich sewage sludge, livestock manures or industrial by-products are applied. Although several estimates derived from models are widely used for atmospheric deposition, it was considered important to measure the amounts of metals deposited and their geographical variation over England and Wales over a 30 month period.

2. Materials and Methods

A network of 35 sites for monitoring total atmospheric deposition was set up in England and Wales using Warren Spring Laboratory-type inverted Frisbees specially moulded from polystyrene (Hall *et al*, 1993). Thirty four of the monitoring sites were at locations remote from major roads and industrial sites but for comparative purposes one was placed near to a known source of pollution, a lead-zinc smelter. The total deposits were collected from the Frisbee collectors on a monthly basis and filtered (0.45μm) in order to provide data for the soluble and insoluble fractions. The filtrate and digested filter residues were analysed for 21 elements by ICP-OES together with Hydride Generation Atomic Absorption Spectrometry for arsenic and Cold Vapour Atomic Absorption Spectrometry for mercury. Samples of soil (0-20cm, 20-40cm and 40-60cm) were taken at each monitoring site and analysed for heavy metals by *Aqua regia* digestion to investigate relationships between atmospheric deposition and soil composition.

3. Results and Discussion

Table 1: Summarised Results for the Atmospheric Deposition of Heavy Metals at Rural Sites in England and Wales (g ha^{-1})

Variable	Mean	CV %	Minimum	Maximum
As	2.93	77	0.86	13.2
Cd	1.83	34	0.86	3.62
Cr	8.68	129	2.22	59.3
Cu	55.9	104	25.7	378
Hg	2.01	314	0.47	37.6
Ni	8.9	71	3.4	40.6
Pb	42.4	34	15.8	92.4
Zn	220	31	111	385

The results in Table 1 show that of all the heavy metals measured in rural areas, zinc was deposited in the greatest quantities (mean 220 g $ha^{-1}a^{-1}$) followed in decreasing order by copper (mean 55.9 g

$ha^{-1}a^{-1}$), and lead (mean 42.4 g $ha^{-1}a^{-1}$). Cadmium deposition was the lowest of the heavy metals monitored (mean 1.83 g $ha^{-1}a^{-1}$) but mercury (mean 2.01 g $ha^{-1}a^{-1}$) and arsenic (mean 2.93 g $ha^{-1}a^{-1}$) were also low. Although barium (not shown in Table 1) is not considered to be a high priority element, the amounts deposited were relatively high (mean 38.3g $ha^{-1}a^{-1}$).

These results are in the same relative range as those reported for Germany by Schulte *et al* (1996) and Schulte and Gerhmann (1996) who reported values of 31 g ha^{-1} for Pb, 2 g ha^{-1} for Cd, 3 g ha^{-1} for As and 3.5 g ha^{-1} for Cr in 1993. Page and Steinnes (1990) give a wider range with values of up to 536 g ha^{-1} for Cd and 1900 g ha^{-1} for Zn but some of these were estimates based on relatively old data.

The data for cadmium, lead and zinc showed the lowest coefficients of variation (31-34%) indicating relatively ubiquitous sources. On the other hand, copper, mercury and chromium showed highly variable and skewed data suggesting a few sources having pronounced local effects. Other data (not presented here) revealed that the soluble and insoluble fractions of the total deposits showed significant variations between elements, sites and times of the year.

In contrast to deposition at remote rural sites, monitoring was also carried out 2.5 km downwind from a large lead-zinc smelter and industrial complex. Deposition at this site for some metals was much higher than the annual mean values obtained from the 34 rural sites. Cadmium deposition was greater by a factor of 30, copper by 2, lead by 35 and zinc by 14.8. These data show that local atmospheric dispersion from a major source such as a smelter can give rise to highly elevated concentrations of potentially toxic elements in the air and soil which could have implications for the health of humans and livestock and crop quality.

Samples of soils from all the monitored sites were analysed for their metal contents but with the exception of the site near to the smelter, there were no significant correlations between the amounts of metals recently deposited and their total concentrations in topsoils (0-20 cm).

4. Conclusions

These results generally confirm the quantities of heavy metals deposited from the atmosphere which have been predicted by models but significant geographical variations can occur which can be important when inventories of metal inputs are being considered for individual sites. The ubiquitous occurrence of zinc and cadmium are noteworthy and their sources require investigation. The major source of lead is still vehicle exhausts but this is expected to decline with the decreasing consumption of leaded petrol.

5. Acknowledgement

This work formed part of a project (OC 9325) "The Vulnerability of Soils to Pollution by Heavy Metals" funded by the UK Ministry of Agriculture, Fisheries and Food (1995-1998) and the work was carried out in collaboration with partners in ADAS and the Water Research Centre, UK.

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DISTRIBUTION OF COPPER IN CHAMPAGNE VINEYARDS SOILS, AS INFLUENCED BY ORGANIC AMENDMENTS.

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1. Introduction

In French vineyards, copper sulfate (Bordeaux mixture) has been used as a fungicide against mildew for more than a hundred years (Delas, 1963). This treatment has resulted in a significant Cu accumulation in soils (from 100 to 1500 mg kg^{-1}). Today, 8 million hectares in the world, 4 in the EC and about 1 in France are concerned by this pollution, even if quantities of Cu inputs have been strongly reduced in the last 20 years.

To study Cu bioavailability and mobility, numerous chemical speciation methods are described in literature but artefacts were currently noted as no selectivity of the chemical reagents, Cu redistribution during extraction (Tack & al., 1993). Recently, physical methods as soil fractionations were described, showing that Cu is dominantly concentrated in the coarse organic fraction, associated with plant residues, and in the clay fraction (Flores-Velez, 1996). The latter results need to be confirmed and even generalized.

In Reuil sur Marne, near Reims (51), vineyards are on steep slopes. Important problems of erosion, especially during thunderstorms, occur (Ballif & al., 1989). Cu is carried away by run-off, inducing an important risk of environmental pollution, in particular for the Marne river. Organic amendments are brought to soils in avoid to limit erosion.

The aim of this study is to analyse the impact of these organic amendments on Cu retention in soils and to identify soil components responsible for Cu retention and of its possible environmental dispersion.

2. Materials and Methods

Surface layers (0-10 cm) of 5 soils were sampled. 4 soils were under vineyard for more than a hundred years. They presented similar agricultural historics, slopes but received different organic amendments (no amendment, vine shoots, cortex, urban compost). The fifth soil, a former wood, was planted under vineyard in 1996 and had never received Cu before soils were sampled.

Samples were analysed for total C and N by elemental analyzer Carlo Erba and total Cu by AAS. Samples were then physically fractionated, without OM destruction, depending on size and nature of separated fractions (Balesdent & al., 1991). The fractionation method combinated a sieving method with densimetric separations in water. Analysis of each organic, mineral and organo-mineral fractions separated allowed to determine Cu distribution in relation to soil components and to components inherited from organic amendments.

Concurrently, aggregate stability of two soils with different organic amendments (unamended soil and soil receiving vine shoots) was investigated (method Le Bissonnais & al, 1996), allowing to

sudy the distribution of Cu in soils aggregates size classes and also permitting measurments of Cu contents in stable and unstable aggregates.

3. Results and Discussion

Samples from soils under vineyard had similar CaCO3 contents (30%) and textures (30% clay, 35% silt). The former wood contained 50% clay.
Samples from soils under vineyard contained 248 to 378 mg Cu kg^{-1} soil. The higher their Organic Matter (OM) content, the higher their Cu content (cortex and vine shoots).
The former wood contained 19 mg Cu kg^{-1} soil in accordance with its origin.

Samples from soils under vineyard had very different Particulate Organic Matter (POM) (i.e vegetal debris > 50 µm) contents according to their organic management, from 1.6% of the total soil for the no amended soil to 7% for the soil receiving cortex. POM's, in particular fine POM 50-200µm were very rich in Cu, until 2500 mg Cu kg^{-1} fraction. When expressing results on a gravimetric basis, the clay fraction displayed the highest Cu contribution.

Results from aggregate stability indicated that soil with vine shoots was more stable than unamended soil. These results confirm the role of OM in soil aggregates stability (Monnier, 1965). Stable small macro-aggregates 200-500µm were found to be the richest in Cu, whereas mico-aggregates 50-200µm had the lower Cu content.

4. Conclusions

In these calcareous soils, we showed that organic amendments affected OM contents, Cu retention and also POM abundance. We also showed the highly significant role of POM and clay fractions in Cu retention. Moreover, it's important to note the preferential but double role of the OM : it limits erosion and accumulates Cu in soils.
We actually identify in situ soil fractions responsible for Cu mobilisation during run-off. In addition, the soil with no amendment and the soil receiving vine shoots were recently equiped with run-off collector systems.

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EFFECT OF LONG-TERM FERTILIZATION ON THE CD AND PB CONTENT OF DIFFERENT SOIL TYPES

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1. Introduction

Since the middle of the century the increased industrial growth, non-ferrous metallurgy, the increasing road and aerial traffic, disposal municipal sewage-water and -sludge and their recycling into the agriculture with other urban wastes and crop by-products have contributed to an undesirable increase of heavy metal concentration in the air, water and soil and, as a consequence to their entry into the human food chain (STANNERS and BOURDEAU, 1995). Through plant uptake from soil and water, heavy metals can get into the plant or by aerial deposition, onto the leaves of freshly consumed vegetables and fruits and so into the animal and human organism (BERGMANN, 1993). The modern analytical methods and high performance analytical equipments allow low detection limits for heavy metals in foods. Numerous experiments prove that heavy metals can cause toxicity in humans and livestock. Lead, cadmium and mercury have the most toxic effects. There have been set tolerable limits for heavy metal contents in food products. Official regulations regarding controls on heavy metal concentrations are of great importance from the point of view of health and environmental protection. It is well-known that Cd content of soils depends also on the quality of parent rock. Cd and Pb occur in greater concentrations in acid soils because their solubility is higher in the lower pH range. Several data from the literature proves that some amounts of Cd can accumulate in soil via repeated P fertilization. Particularly the use of superphosphates produced from phosphate rocks originating from Africa must be controlled to minimize heavy metal concentrations. Phosphate rocks originating from Togo, Morocco, Mexico and Tunisia have the greatest concentration of cadmium. Lead (Pb), however, endangers our environment first of all through recycling industrial wastes and through vehicle emissions. Long-term fertilization experiments are the most reliable means to follow the process of soil pollution, accumulation of Cd and Pb in soils as a function of fertilization. In Hungary there has been maintained a network of long-term fertilization experiments (National Long-Term Fertilization Trials) at nine different agro-ecological sites of the country for 28-30 years.

2. Materials and Methods

The experiments were set up with four-year crop rotations of winter wheat-maize biculture in four repetitions in 1967-69. The following fertilizer rates were uniformly applied at all sites but not in every combinations: 0-50-150-200-250 kg N/ha/year, 0-50-100-150-200 kg P_2O_5/ha/year, 0-100-200 kg K_2O/ha/year. Detailed description of the experiments are given by DEBRECZENI and DEBRECZENI (1994). After the 7th rotation (i.e. in the 28th experimental year) we examined the effect of increasing fertilizer doses on the accumulation of Cd and Pb in he upper soil layer of 20 cm. Plant available Al-soluble P_2O_5 and 0.025M EDTA-soluble Cd and Pb concentrations were determined.

The main soil characteristics of the experimental sites:

1 / Iregszemcse, Calcaric phaeosem (calcareous chernozem soil), 22% clay, pH(KCl) = 7.4
2 / Keszthely, Eutric cambisol (lessivated brown forest soil), 24% clay, pH(KCl) = 6.3

3 / Bicsérd, Luvic phaeosem (chernozem brown forest soil), 33% clay, pH(KCl) = 5.4
Fertilization treatments:
1/ unfertilized control
2/ 100 kg N/ha 50 kg P_2O_5/ha, 100 K_2O kg/ha
3/ 150 kg N/ha, 100 kg P_2O_5/ha, 100 K_2O kg/ha
4/ 200 kg N/ha, 150 kg P_2O_5/ha, 100 K_2O kg/ha
5/ 250 kg N/ha, 200 kg P_2O_5/ha, 200 K_2O kg/ha

3. Results and Discussion

As a result of the long-term effect of 28 years of fertilization, considerable available P amounts (Al-soluble P_2O_5) accumulated in the upper 20 cm soil layer of the tested experimental sites. In addition to the P-amounts harvested by winter wheat and maize plants, 2.5-4.6-fold more P could be detected in the soils of the fertilized plots as compared to that of the unfertilized ones. The long-term effect of increasing P doses on the available P content of soils was different at each experimental sites. The extent of P accumulation was the lowest on calcaric chernozem soil (Iregszemcse), the highest on chernozem brown forest soil (Bicsérd) and moderate on clay lessivated brown forest soil (Keszthely). Unlike the P accumulation in soil, grain yield increases showed a reverse order. Remarkable yield increases could be detected at Iregszemcse, moderate ones at Keszthely, while at Bicsérd, P fertilization did not result in yield increases every experimental year. The yield increasing effect was much larger with maize than with wheat.
As regards the tested heavy metals, only slight concentration increases could be detected and mainly for Pb in the soils of sites involved in the present study. Cd concentrations amounted to only 0.10-0.13 mg/kg, while Pb concentrations to 2.4-10.4 mg/kg. Data from the literature show that the amount of heavy metals, among them that of Cd and Pb, originating from aerial deposition can exceed the amounts accumulating by P fertilization in soils. Pb contamination of soils is mainly caused by deposition from gases emitted by road traffic. Keszthely is the site that has the heaviest traffic among the three sites involved in the present investigations. In addition, the experimental area is nearer to the highway at Keszthely than at the two other sites. As it was to be expected, the highest Pb amounts were measured in its soil samples, 8.3 mg/kg even in the soil samples of the unfertilized control plots. Clay content and acidity of the experimental soils showed a close relationship with Cd accumulation. P accumulation in the soils affected Cd accumulation too. The analytical data for EDTA-soluble Cd are below the tolerance limit, although they show a slight increase as an effect of increasing P doses. The differences in Cd accumulation detected between the tested soil types were greater than those effected by different doses of P fertilizer.

4. Conclusions

Luckily, the environmental impact of Cd and Pb resulting from the P fertilization over 28 years can not be considered as remarkable at the tested experimental sites up to the present time. Unfortunately, we have no analytical data on the initial heavy metal content of soils at the experimental sites, since less attention was given to fertilizer impact on environment at the time when the network of the trials was established than today. In addition to soil analysis for heavy metals, chemical analysis of crop products may also be needed when investigating the undesirable environmental impacts of fertilization in the future.

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ENVIRONMENTAL TRANSFER OF COPPER IN CALCAREOUS AGRICULTURAL SOILS

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key words: copper, heavy metal contamination, calcareous soils, speciation study, metal transfer.

1. Introduction

A study on the mobility of copper in calcareous soils near an old mining site in semi-aridic conditions (S.E. Spain) is presented. The mobility of both solid particles and soluble compounds of copper are considered. The process governing copper transfer from the element-rich zone to the neighbouring soils are strongly influenced by the semi-aridic climate, being the presence of high proportion of carbonates an important factor in the mobilization of the metal. The soils studied are calcaric fluvisols developed from Quaternary alluvial sediments from the sorrounding hills, some areas of which are dedicated to mining. The slag heaps, which are mixtures of mining wastes, mineral and rock, have undergone a process of supergenic alteration to give rise to secondary minerals such as kaolinite, smectite, alunite, jarosite, gypsum and a number of polyhidrated complex soluble sulphates of Zn, Mg, Fe, Mn, Na, and K, such as copiapite, halotrichite, botryogene, bianchite... These form soil solution and incorporate smaller quantities of Ag, Ge, Sn, Pb, Cu, ...(1).

2. Materials and Methods

Ten soil samples were obtained from a 5 km^2 sampling zone. In addition, five samples were obtained from the main gully and thirty from the slags. The general characteristics of the arable soil layer were detremined by the methods used to draw up a soil map of the area in the LUCDEME proyect (2). The total metal content was determined by electrothermal atomization atomic absorption spectrometry (ETAAS) or flame atomic absorption spectrometry (FAAS). For this, the samples were slurried in a dilute hydrofluoric acid solution and the suspensions were directly introduced into the flame (3) or the electrothermal atomizer (4).

The selective extractants used were: water; 1M ammonium acetate at pH 7.0 (5); 0.5 M ammonium acetate + 0.02 M EDTA + acetic acid at pH 4.65, ; 0.1 M sodium pyrophosphate, oxalic acid-oxalate (6) ; DTPA (7) ; sodium dithionite + sodium citrate (8) ; HNO_3 10% vv. In addition, an accepted sequential extraction procedure (9) based on three successive extractions stages was also applied to the same samples and the results were compared with those found using the above mentioned reagents. The metal content in each fraction was analyzed by ETAAS or FAAS. The mineralogical composition of the samples was determined by XRD analysis using radiation Cu-Kα. Using the diffraction intensities obtained and the values for the same phases given in the bibliography, a semiquantitative analysis was made of the treated and untreated samples. The samples were also examined using scanning electron microscopy (SEM) instrument equipped with an EDS analyzer.

3. Results and Discussion

The extraction values in H_2O (metal extracted x 100 / total content) point to the natural inmobilization of copper when it is incorporated in carbonated soils, although these values diminish as getting further from the source. The nitric acid extraction medium is a more

aggressive medium than the mine acid waters and permits the solubilization of the jarosite and alunite of the slags and their transportation to adjacent agricultural lands in the form of particulate materials. The incorporation of these minor quantities of copper to the soils is the consequence of three sucessive stages. The first step is the supergenic alteration process suffered by the mining wastes, which are sulphide-rich, to give rise to soluble sulphates. Next, because of occasional but torrential rainfalls, these sulphates are leached both as soluble compounds and as particulate materials. Finally, these acidic sulphate–rich lixiviation products are neutralized by the high proportion of carbonate present in the soils, being the copper in this way immobilized in the soils. The results obtained by using the selective extractants agreed with the data obtained from the XRD and SEM studies of both the original samples and the residues remaining after each extraction stage. Our data indicate that when dealing with samples with a high carbonate content, which are the representative of aridic soils present in wide zones of the Mediterranean Basin, neither the sequential nor single extraction aproaches provide a suitable extraction scheme. A reliable assessment of metal availability in these highly calcareous soils must take into account the mineral phase in which the copper is present.

Table 1 Mean values of copper for all samples studied (mg/kg)

	SLAG			GULLY			SOIL		
	total	H_2O Ext %	HNO_3 Ext %	total Ext %	H_2O Ext %	HNO_3 Ext %	total Ext %	H_2O	HNO_3
Cu	650	20	60	230	0.4	10	180	0.3	25 (mg/kg)

Table 2 Mean values obtained for the soil samples using the selective extractions (soluble x100/total) and sequential procedure (three stages)

	1stage	2stage	3 stage	NH_4Ac	EDTA	DTPA	Oxal.	Cit.-Dit.	Pyroph.
Cu	10	8	12	1.2	1.9	14.0	9.0	19.0	4.4

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DETERMINATION OF MN AND CU EFFICIENCY OF CROP PLANTS IN POT EXPERIMENTS

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1. Introduction

Several studies of manganese (Mn) and copper (Cu) efficiency are reported in the literature (Bergmann, 1992). However, most experiments include only a few plant species and it is unclear, how efficiency is defined: in terms of decreased yield or the development of deficiency symptoms. Our aim was to determine the Mn and Cu efficiency on the basis of yield response of a large range of crop plants under identical experimental conditions.

2. Materials and Methods

Pot experiments were conducted with either a Mn or a Cu deficient peat soil (4 kg pot^{-1}). The Mn concentration of the soil (sulfite pH 8 method Schachtschabel) was 4 g and the Cu concentration in the soil (0.43 M HNO_3) was 0.2 g, always per m^3 of undisturbed soil. The soils of the control pots received before planting sufficient Mn (6 g pot^{-1}) or Cu (200 mg pot^{-1}). For each crop species, yield of the main harvest product was determined. Efficiency was expressed in terms of yield, thus including both uptake and utilization efficiency and was defined as yield at low supply relative to yield at sufficient supply of the soil (see tables).

3. Results and Discussion

The cereals had the strongest yield decrease and thus the lowest **Mn efficiency** of all crop plants tested (table 1). Under Mn deficiency, grain yield was only 14 (wheat) to 44 % (rye) of that with sufficient Mn supply. Mn efficiency increased in the following order:
Wheat < oats < barley < rye < ryegrass < alfalfa < maize(silage) = cauliflower < spinach = common bean < spring rape = red clover < potato = sugar beet = tomato < fodder beet.

The lowest **Cu efficiency** was found in flax and wheat, where yield of plants with low Cu supply was only 19 and 20 % of the yield under Cu sufficient conditions, respectively. A strong yield decrease and thus a low Cu efficiency was also found in barley, maize(silage), oats, alfalfa and spinach. On the other hand, ryegrass and rye had the highest Cu efficiency. Cu efficiency increased in the following order (table 2):
Flax < wheat < barley < maize(silage) < oats < alfalfa < spinach < fodder beet < sugar beet < red clover <maize(grain) < potato < Brassica napus L. var. napobrassica < spring rape< rye < ryegrass.

4. Conclusions

The results are in agreement with other studies. The method of testing a range of plant species under identical conditions is also appropriate to determine efficiency for other nutrients.

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Table 1: Determination of Mn efficiency of crop plants

Crop	Harvest product	Yield: Mn supply of soil sufficient		Yield: Mn supply of soil low		Mn efficiency[1)]
		g pot^{-1}	relative	g pot^{-1}	relative	
Wheat	grain	77.4	100	10.8	14	low
Oats	grain	102.1	100	20.4	20	
Barley	grain	82.0	100	34.4	42	
Rye	grain	82.6	100	36.3	44	
Ryegrass	3 cuttings DM	88.9	100	57.0	64	medium
Alfalfa	3 cuttings DM	92.3	100	60.9	66	
Maize	shoot DM	175.0	100	119	68	
Cauliflower	flower FM	246.0	100	167.3	68	
Spinach	shoot DM	13.5	100	11.3	84	
Common bean	husk DM	40.6	100	34.5	85	
Spring rape	grain	40.5	100	36.4	90	
Red clover	2 cuttings DM	49.0	100	44.6	91	
Potato	tuber FM	398	100	397	100	high
Sugar beet	beet FM	503	100	528	105	
Tomato	fruit FM	1160	100	1218	105	
Fodder beet	beet FM	674	100	775	115	

[1)] corresponds to relative yield under low Mn supply of the soil

Table 2: Determination of Cu efficiency of crop plants

Crop	Harvest product	Relative yield at low soil Cu supply (100 = yield at sufficient Cu supply)			Cu efficiency[1)]
		Experiment 1	Experiment 2	Mean	
Flax	straw	16	22	19	low
Wheat	grain	30	11	20	
Barley	grain	54	46	50	
Maize	shoot DM	63	54	58	
Oats	grain	50	70	60	
Alfalfa	3 cuttings DM	48	76	62	
Spinach	shoot DM	56	76	66	
Fodder beet	beet DM	80	60	70	medium
Sugar beet	beet DM	70	76	73	
Red Clover	3 cuttings DM	78	80	79	
Maize	grain	88	78	83	
Potato	tuber FM	90	88	89	high
Brass. napobrass.	beet FM	94	86	90	
Spring rape	grain	-	93	93	
Rye	grain	87	104	96	
Ryegrass	3 cuttings DM	94	102	98	

[1)] corresponds to relative yield under low Cu supply of the soil

THE ACCUMULATION AND LEACHING OF FERTILIZER-DERIVED CADMIUM IN A NEW ZEALAND PODZOL SOIL

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1. Introduction

Animal production on legume-based pastures is a major contributor to export revenue in New Zealand. Productive pastures are sustained by annual inputs of phosphorus (P) and sulfur, mainly in the form of single superphosphate (SSP) - the application rate varies but is usually 100-500 kg SSP/ha. Like most phosphatic fertilisers, SSP contains undesirable elements that may accumulate in the soil (McLaughlin *et al.*, 1996). One such element is the potentially toxic heavy metal cadmium (Cd). In order to assess the risk associated with Cd accumulation in soil, information is required on the residence time of Cd in, and the factors affecting its loss from topsoils. This paper examines the accumulation and loss of fertiliser-derived Cd in a New Zealand podzol as a function of time and changing soil chemical properties.

2. Materials and Methods

Soil samples at depths of: 0-3 cm; 3-7.5 cm; 7.5-top of E (eluvial) horizon were taken from 7 sites located on a podzol soil in northern New Zealand – the Wharekohe silt loam (Edwards, 1997). Five of these sites had been under pasture and receiving known rates of SSP for 8, 23, 25, 30 and 32 years respectively (Edwards, 1997). The other 2 sites were undeveloped (0 years fertiliser addition). Bulk densities and pH were recorded and the soils analysed for total phosphorus (sulphuric, nitric and perchloric acid digestion) and total Cd (HF and nitric acid digestion). Pasture samples were also collected and analysed for total Cd (nitric acid digestion). Cadmium concentrations in the digest solutions were determined by graphite furnace atomic absorption spectrometry (GFAAS).

In laboratory-based leaching experiments, two soil samples taken from the 0 year (Undeveloped) and 25 year (Developed) sites were treated with 1 $\mu g\ g^{-1}$ Cd (as $Cd(NO_3)_2$). These were repacked into columns (45 mm diameter) and leached with 2.5 mM $CaCl_2$. The leachate samples were analysed for pH and Cd (by GFAAS). At the completion of leaching, the soil columns were segmented and each segment analysed for Cd (nitric/hydrochloric acid digestion; GFAAS).

3. Results and Discussion

Regular SSP application resulted in Cd accumulation in the surface soil. Mass balance calculations, however, indicated that considerable quantities of Cd (up to 44%) had been lost from the sampling zone at 4 of the 5 sites (Figure 1). Loss of applied P from this podzol is also known (Edwards, 1997). Other studies also report Cd losses from pasture soils of between 40 and 50% (Rothbaum *et al.*, 1986; Nicholson *et al.*, 1996). Cadmium uptake by plants and removal in animal products could not account for this loss and the sites were undisturbed and not impacted by lateral soil movement or erosion. The primary means of Cd loss from the sampling zone was probably leaching. Annual leaching rates of between 4.6 g Cd/ha and 7.7 g Cd/ha were calculated. The feasibility of leaching as the major loss

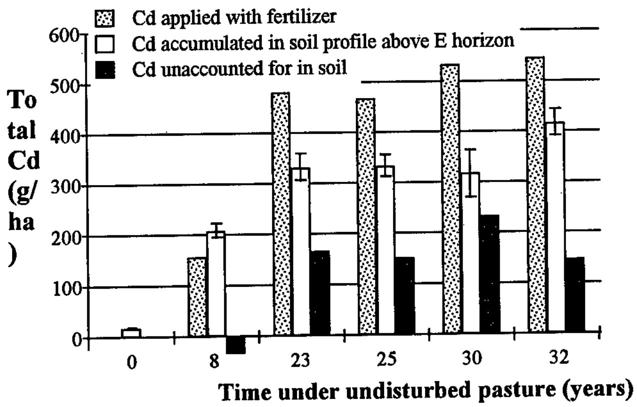

Figure 1. Cadmium mass balance calculated for each site. Error bars denote the standard error of the mean value for at least four pits.

mechanism was confirmed in column leaching experiments. Cadmium mobility in the Wharekohe soil was highly variable. A model developed from the leaching data predicted annual leaching rates of 75 g Cd/ha for the Undeveloped soil and 1.4 g Cd/ha for the Developed soil, given a topsoil Cd concentration of 0.44 mg/kg. This difference was attributed to the increase in soil pH and carbon content brought about by lime and superphosphate application during pasture development. While the Undeveloped soil had a pH of 4.5 and carbon content of 4.8%, the Developed soil had a soil pH of 6.0 and carbon content of 7.5%. The lower rate of Cd leaching in the Developed soil suggests that Cd losses from the Wharekohe podzol would be greatest under newly established pasture, when the application of large quantities of superphosphate would coincide with a very low soil pH.

4.Conclusions

Despite strong accumulation of Cd in the surface soil, the Wharekohe silt loam has experienced substantial losses of Cd. These losses appear to have occurred through leaching, principally during the early stages of pasture development when soil pH was still low.

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CADMIUM AND ZINC FLUXES AND BALANCES IN ORGANIC AND CONVENTIONAL DAIRY FARMING - PRELIMINARY RESULTS

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1. Introduction

One approach to evaluating the sustainability of different farming systems is to determine how they affect elemental balances on a field scale. By quantifying different pools and fluxes, any imbalances can be detected, and changes in concentrations and quantities of important elements in the soil system can be estimated (Andersson 1992). One of the long-term goals of the Swedish Environmental Protection Agency is to ensure that there is no net accumulation of heavy metals in agricultural soils. Within the "FOOD 21-Sustainable Food Production" program (Bylund et al 1997) trace element research is being carried out at the Öjebyn Experimental Farm where conventional and organic dairy farming have been practised side by side since 1988 (Fagerberg et al 1996). In this paper the soil pools of Zn and Cd are quantified, and preliminary input-output budgets are calculated.

2. Materials and Methods

Öjebyn Farm is situated in north-eastern Sweden (65°21'N 21°24'E) on the coast of Bothnian Bay where the soils remain frozen about 6 months per year, resulting in a high proportion of surface runoff. The farm has 100 ha of agricultural land and 100 dairy cows which are divided between the two farming systems. The crop rotation is: forage crop (oats and peas) with undersown ley - three years of ley - barley - potatoes/forage crop. All crops are represented each year. The farm survey includes a total of 57 fixed sampling sites evenly distributed over the area where crops are regularly harvested and soil samples have been taken. Manure, urine and mineral fertiliser are sampled at the time of application. Soil water is collected with suction lysimeters at depths of 25, 50 and 75 cm in the two major soil types (Eutric Regosols and Thionic Gleysols), which occur in both farming system areas.

Input-output budgets were calculated according to the principles used by Andersson (1992). Analyses of soil water chemistry at 25 cm were used to quantify losses via surface runoff, and the value at 50 cm was used to estimate leaching from the root zone. Preliminary budget calculations are based on one year's field measurements.
Inputs: Manure + Urine + Mineral fertilisers + Lime + Atmospheric deposition
Outputs: Harvest + Drainage water + Surface runoff water

3. Results and Discussion

Table 1 shows the Cd and Zn concentrations and the pools in the soil horizons. Cd and Zn contents were significantly higher in all soil horizons in the organic system. This was probably due to an uneven distribution of soil types between the two farming systems or to differences in farming practise before the organic farming was introduced.

Table 1. Average Cd and Zn contents (7M HNO_3, digested 2h) in three soil horizons for the organic (Cd_o, Zn_o, *n=26*) and conventional (Cd_c, Zn_c, *n=22*) farming systems

Depth (cm)	Soil content (mg kg^{-1})				Soil pools (kg ha^{-1})			
	Cd_o	Cd_c	Zn_o	Zn_c	Cd_o	Cd_c	Zn_o	Zn_c
0-25	0.15	0.11	44	35	0.35	0.24	116	88
25-55	0.05	0.03	43	31	0.21	0.11	167	118
55-85	0.05	0.03	45	31	0.20	0.10	166	120

The input-output budget calculations show that manure and atmospheric deposition are the main Zn and Cd sources (Table 2). The trace element content was significantly higher in the manure from the conventional system. Purchased feed concentrates could have been one important source of these elements. The removal of Cd in runoff water was the most important output. For Zn, removal at harvest was the major output. The budget shows that the amounts of Cd and Zn added to the soil system in the form of mineral fertilisers are negligible today. The calculations indicate that there was a small increase in Cd in the conventional system but no change in the organic one. Furthermore, the budget indicates that Zn accumulated in both farming systems, but the rate was twice as high in the conventional one.

Table 2. Preliminary estimates of the annual average inputs and outputs of Cd and Zn (g ha^{-1}year^{-1}) over one crop rotation for the organic (Org.) and conventional (Con.) farming systems. NOTE: The calculations are based on one year's measurements

	Inputs					Outputs			Differ-ence	% change[1]
							Water runoff			
	Manure	Urine	Ferti-liser	Depo-sition	Lime	Harvest	Drainage	Surface		
Cd										
Org.	0.27	0.01	0	0.45	0.02	0.28	0.23	0.30	-0,06	-0.01
Con.	0.40	0.04	0.01	0.45	0.01	0.21	0.23	0.30	+0.17	+0.05
Zn										
Org.	405	12	0	85	2	184	23	49	+248	+0.09
Con.	494	38	8	85	1	180	23	49	+374	+0.18

[1] Annual change in soil pool calculated as difference/soil pool (0-55 cm)*100

4. Conclusions

Inputs of Cd and Zn were lower in the organic farming system, mainly owing to the higher input via manure in the conventional system. The purchased feed concentrate could be one possible source of the higher manure metal contents in the conventional system.

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DOES VINEYARD CULTIVATION AFFECT COPPER ACCUMULATION IN SOIL ?

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1. Introduction

The vineyard is the most affected cultivation by the use of copper which is widely used for its protection, either alone (Biological Management) or mixed with some other anti-peronosporaceous (Integrated Management). In a recent work (Moolenar and Beltrami, 1998) it has been estimated that the environmental sustainability of copper applications in vine-growing is by itself very low and it is 100% lower when biological protection is compared to the traditional integrated protection. In the case of a biological vineyard, defended only with cupric products (26.2 kg/ha/year), the estimated necessary time needed to reach the intervent value (100 ppm) is of 13 years value; in those vineyards where copper is applied and mixed with other fungicides (10.5 kg/ha/year) it takes 31 years to reach this value.
The aim of this paper is to evaluate the present soil accumulation of copper in vineyard areas in Italy and to identify the anthropic and pedogenic cause.

2. Materials and Methods

Representative wine production areas have been selected in different Italian regions. Liguria (Pigato wine), Trentino (Teroldego), Veneto (Amarone), Emilia-Romagna (Lambrusco, Gutturnio and Monterosso), Campania (Greco) e Sicilia (Regaleali). In each area a few farms have been identified having homogenous soils and species of vine with different ages protected by biological or integrated pest management. A blank soil that has never been cultivated with grapes in the last 50 years has also been monitored.
In each soil all the informations regarding the type of soil, crop management, agronomic practice and history of the pesticide treatments have been collected. The soil was then sampled per each horizon of the pedological profile and copper content analysed by ICP after extraction in Aqua Regia.

3. Results and Discussion

The obtained results demonstrate that the majority of Italian soils cultivated with vineyard exceed the intervent value. Copper accumulations are evident above all in the surface layers of the soil and this is due to the adopted pest management. Crops with biological management always have larger contamination levels than the integrated ones, but in some conditions the contamination level is higher than the intervent value and this is because of a pedogenic origin. An example of copper distribution in the soil profile of typical italian vinegrowing areas is illustrated in table 1.
Finally, some considerations can be done. EU legislation on biological farming as well as the legislation on environmental protection against metals should be reviewed trying to find the inconsistencies which cause disparity in the use of farming soils bringing repercussions on the farm balance. The intervent value for copper in vineyard soil should be reconsiderated taking into account the present level of contamination. New pest management strategies including the development of new formulations with copper and organic pesticide should be adopted in order

to reduce the copper dosage. Sustainability criteria for the use of copper should be linked to the soil characteristics and to the intrinsic value of the wine area.

Table 1 – Copper distribution (range) in the soil profile of two italian wine area (mg/kg dry soil). Biological management (BM) and Integrated management (IM) with different time range.

Horizons (cm)	*Lambrusco area*		
	BM (15 years)	**BM (30 years)**	**Grass (50 years)**
0-30	86.7÷102.6	117.3÷172.0	68.2÷83.4
30-50	63.4÷95.6	111.3÷51.3	20.3÷23.3
50-100	34.3÷51.6	24.5÷32.7	16.7÷23.4
	Teroldego area		
	IM (29 years)	**IM (3 years)**	
0-30	200.0÷247.7	199.7÷251.0	
30-50	79.3÷253.0	61.6÷115.8	
gravel	70.5÷86.2	47.4÷75.8	

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EFFECTS OF ACIDIFICATION AND IRON ENRICHMENT OF CALCAREOUS SOILS ON PLANT NUTRITION

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1. Introduction

Many crops grown on calcareous soils exhibit Fe deficiency symptoms and are variably affected in their development. The Fe deficiency chlorosis in plants grown on these soils is not induced by actual Fe deficiency in the soil but rather a combination of high soil pH, $CaC0_3$ and $HC0^-_3$ in the root rhizosphere, and Fe immobility in the plant caused by increased pH levels in the leaf apoplast. Grain sorghum (Sorghum bicolor(L)Moench) has a relatively low use efficiency of soil Fe and readily exhibits Fe deficiency symptoms (Matocha,1984). Field observations have noted an apparent association between Fe deficient, chlorotic grain sorghum and the incidence of Phymatotrichum root rot (PRR) on cotton(Gossypium hirsutumL.) grown in alternate years. Thus, a study of a possible relationship between trace element nutrition and cotton plant susceptibility to the pathogen was made. Additional studies were conducted to correct Fe deficiency on grain sorghum by manipulating the soil rhizosphere pH and levels of plant-available Fe.

2. Materials and Methods

Acid, acid forming fertilizers, synthetically chelated Fe sources (FeEDDHA) and Amaranthus plant-complexed Fe material were evaluated for their effectiveness in reducing Fe deficiency chlorosis and influencing plant growth of two important agronomic crops in Texas, grain sorghum and cotton. Treatment response parameters for grain sorghum included, dry matter yield, Fe concentration and uptake. With cotton, barnyard manure was included as a supplier of Fe and organic matter while treatment evaluations included plant mortalities from the soil borne Phymatotrichum Omnivorum disease (PRR).

The cotton experiment was conducted on a Clareville clay loam (Pachic Argiustoll) while the grain sorghum tests were performed on the same Clareville clay loam and an Orelia clay loam (Typic Ochraqualf). The soils for the cotton test were selected from a field with a Fe deficiency chlorosis on grain sorghum and moderate to severe pressure from PRR on cotton Matocha and Hopper, 1995). For the sorghum test, soils was selected from field sites where sorghum exhibited Fe deficiency chlorosis and also sites with nonchlorotic grain sorghum. These paired analogues were then treated in laboratory analyses and evaluations in greenhouse experiments.

3. Results and Discussion

Soil response in the greenhouse grain sorghum tests one week following treatment application showed H_2S0_4 acid amendments prior to planting increased plant-available, DTPA extractable Fe by 100-200 fold while the addition of chelated Fe at 4 mg kg^{-1} increased extractable Fe 2-3X. Following the growth of grain sorghum for six weeks, chemical estimations of plant-available Fe showed reductions of 23 and 76 percent, respectively, for the acid and chelate treatments. At the completion of the second sorghum crop, an additional seven weeks time, the soil Fe levels corresponding to the same treatments were reduced by 63 and 68 percent. The actual soil Fe values had decreased for the Clareville soil from 513 to 191 and 7.1 to 2.3 mg Fe kg^{-1} soil for the H_2S0_4 acid and Fe-EDDHA treatments, respectively. Comparable readings for the Orelia

soil were 411 to 16 and 6 to 3.8 mg Fe kg^{-1} soil, respectively for H_2SO_4 acid and Fe-EDDHA. Soil pH readings for the corresponding sampling periods on the Clareville soil were: untreated 7.7; acid treated 2.4 and 12 weeks later untreated 7.0; acid treated 3.3.

Grain Sorghum: Measurements of grain sorghum response to soil treatments were limited to chelated Fe since the acid treatment caused elevated salinity in the seeding zone which prevented seedling emergence. Plant growth was negligible in Fe-control soil due to severe chlorosis but increased 2700 percent on the Clareville soil and 600 percent on the Orelia soil due to an application of 4 mg Fe kg^{-1} as chelated Fe (Moseley, 1996). Treatments on the nonchlorotic paired samples of each soil resulted in yield increases of 270 and 210 percent, respectively.

Cotton: Granular elemental S at 8,000 and 16,000 kg ha^{-1} increased dry matter yields of cotton (76-164% over control) and decreased plant mortalities from PRR. Powdered elemental S was not as effective in suppressing the disease and improving plant growth compared to granular elemental S. Barnyard manure increased plant growth and had a moderate effect on PRR suppression. Plant uptake and tissue Fe varied with treatment and were in the following order: H_2SO_4>granular elemental S=powdered elemental S. In another study, cotton did not exhibit chlorosis but rather a pale green color. These plants responded to FeEDDHA, a mine byproduct Fe source and Amaranthus plant-complexed Fe. Generally, 5 to 10 mg Fe kg^{-1} soil of chelated or plant-complexed Fe sources equaled 200 mg Fe kg^{-1} as inorganic byproduct sources in their effects on growth and PRR suppression. These data suggests that improving the rhizosphere levels of available Fe and nutrition of the cotton plant can result in reducing severity of PRR.

4. Conclusions

Results of these studies have shown that soil amendments such as acidification with acid and acid base fertilizers or addition of synthetically developed Fe chelates and plant complexed Fe materials are effective in improving the plant-available Fe status of alkaline-calcareous soils. These practices reduce Fe deficiency chlorosis on grain sorghum and suppress the incidence of PRR disease on cotton.

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EVALUATION OF CRITICAL LIMITS OF TRACE ELEMENTS ZN, B AND MO IN BANGLADESH AGRICULTURE.

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1. Introduction

Recently greater attention has been paid to the evaluation of micronutrient status in crop production in Bangladesh. Experimental results and agricultural practices have indicated that the areas deficient in trace elements are increasing in Bangladesh due to intensive cultivation of HYV crops and modernization of agriculture. Therefore, a systematic and detailed appraisal on the status and distribution of trace elements is essential for correct application of micronutrient fertilizers. With the data available to date, an evaluation of critical limits and related problems on Zn, B and Mo are discussed in this paper.

2. Materials and Methods

Representative soil samples were collected from different agroecological zones of Bangladesh. Soil analyses were done by DTPA extraction for zinc, calcium biphosphate extraction for boron and NH4 -exalate extraction for molybdenum. Plant responses data of greenhouse experiments of different soil series were compiled, screened and processed by computer to evaluate the critical limits of Zn, B and Mo in soils.

3. Results and Discussion

Zinc - Available Zinc was extracted by DTPA extractant and determined by atomic absorption spectrophotometry method. Available Zn is divided into six categories based on an updated critical limit 0.6 μg/g by.

LEVEL OF ZN	QUANTITY IN µG/G	LOCATION/AEZ
Very low	<0.45	Ganges Floodplain, Active Tista Floodplain, Hill soils, High Ganges River and Ganges River Floodplain
Low	0.451 - 0.9	Old Himalayan Piedmont plain, Tista Floodplain,Chittagong Coastal plains, Brahmaputra Floodplain, Ganges Tidal Floodplain, Meghna River Floodplain
Medium	0.91 - 1.35	Atrai basin, Old Brahmaputra Floodplain, Surma-Kushyiara Floodplain, Barind Tracts, Akhaura Terrace, Madhupur Tracts.
Optimum	1.351 - 1.8	Gopalgong-Khulna Bill, Atrai Basins,Northeast Barind Tract
High	1.81 - 2.25	Arial Bils, Atrai Basin, Sylhet Basin, Peat soils
Very high	>2.25	Sylhet basin, Acid Basin Clays

Soil conditions associated with Zn deficiencies in Bangladesh are: acid sandy soils low in total Zn, calcareous soils and saline soils.

Boron - The available B in the soil was determined by Ca-biphosphate extractent and spectrophotometric method. The available B content of all the major soils of Bangladesh

varied between 0.1 and 1.9 µg/g in the surface layer. Most of the light textured soils of Rangpur and Dinajpur, Terrace soils of Gazipur and Hill soils of Srimangal and Ramgahr were found deficient in boron (0.1 - 0.2 µg/g) based on critical limit 0.2 ug/g. Boron content in soils was found to be positively correlated with clay content and available S.

Molybdenum - The available Mo content (NH4 - oxalate extractant) of some major soils ranged between 0.03 to 0.43 µg/g. The soils of Rajshahi, Rangpur, Dinajpur and Bogra locations contain low level of Mo (0.03 - 0.08 µg/g). based on critical limit 0.1 µg/g..

4. Conclusions

Chemical analysis, fertilizer experiments and field surveys have proved that there is a large area of soils low in micronutrients in Bangladesh. The deficient areas are likely to increase in the future with further crop, intensification. Therefore a systematic study is needed for clear delineation of micronutrient deficient areas in Bangladesh.

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DIFFERENCES IN THE ASSIMILATION OF LEAD AND CADMIUM BY VEGETABLES CULTIVATED UNDER TRADITIONAL AND ECOLOGICAL CONDITIONS

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1.Introduction

Plant uptake is one of the major pathways for potentially toxic metals to enter the food chain. Consequently, interest has aroused during the last years in the use of cultivations under ecological conditions, in this way decreasing the risk for animal and human health. This communication reports our studies on the accumulation of cadmium and lead in broccoli. The plants were cultivated in soils with a high carbonate content using both traditional and ecological agronomical practices.

2.Materials and Methods

Forty soil samples were used in the study. Twenty of these samples corresponded to soils submitted to traditional cultivation practices whereas the rest were obtained from soils with the same characteristics but in which ecological cultivations wereused. Samples of the water used for irrigation of both types of cultivations were also obtained and analyzed. Samples of the plant material (broccoli) were obtained at different stages of growing.
The total metal content (Pb and Cd) was determined by electrothermal atomization atomic absorption spectrometry (ETAAS)[1]. To this end, the soil samples were slurried in a dilute hydrofluoric acid solution and the suspensions were directly introduced into the electrothermal atomizer. The vegetable samples were slurried in a mixture of nitric acid and hydrogen peroxide, the suspensions being also directly introduced into the electrothermal atomizer[2].
The bioavailability of Cd and Pb in the samples was studied by means of the selective extraction methodology, using DTPA[3] and $NaHCO_3$[4] as the extractants.

3. Results and Discussion

Table 1 summarizes the physical-chemical properties of soils where broccoli was grown. Soils used in the experiment do not present serious differences in physical-chemical properties nor in Pb and Cd contents.

Table 1. Selected physical-chemical characteristics of soils.

	% O.M	pH		% clay	E C(ds/m)	% $CaCO_3$
		H_2O	KCl			
Traditional conditions	2.3	7.8	7.5	24.5	2.7	44.5
Ecological conditions	1.1	7.7	7.4	26.7	1.9	53.2

Table 2 presents the total Pb and Cd contents in soils. In any case, these values were below those quoted as the maximum permissible in the current European Union regulations (CEC, 1986)[5].

Table 2. Average metal contents in soil and mean value obtained in the selective extractions (solublex100/total).

	Total (ppm)	DTPA	$NaHCO_3$
		Pb	
Traditional	34-45	10	1
Ecological	18-31	4	1
		Cd	
Traditional	0.5-1	<0.1	<0.1
Ecological	0.5-3	<0.1	<0.1

In general, the ability of the extractants to predict metal availability to plants depends on the crop species, the metal, the extractant used and the soil type[6,7]. As Table 2 suggests, the most reliable reagent for the assessement of the plant uptake in calcareous soils is the DTPA test. The mobilisation of Pb and Cd obtained when $NaHCO_3$ was used as the reagent provided values lower than those existing in reality. These extractants were better predictors for Pb and Cd when these metals were on carbonated soils.
Table 3 demonstrates that broccoli accumulated more Pb than Cd in both types of samples. Pb uptake in the traditional cultivations conditions was increased with respect to ecological conditions. However, significant differences ($p<0.01$) were not found in the case of Cd. The transference of lead from soil to plant is higher during the final stages of growing.

Table 3. Soil to plant transfer coefficients (transfer ratio = plant metal concentration x100/soil metal concentration).

Element	Traditional soils		Ecological soils	
	Initial growing step	Final growing step	Initial growing step	Final growing step
Pb	2.5	4.8	2.1	3.5
Cd	<0.1	<0.1	<0.1	<0.1

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MICROELEMENTS CONTENT IN AGRICULTURAL SOILS OF CENTRAL ITALY: A COMPARISON AFTER EIGHT YEARS

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1. Introduction

Knowledge of microelements content in intensively cultivated alluvial soils of Central Italy is still incomplete. In previous studies, we used a multivariation statistical approach to study the distribution of some heavy metals in the Rieti plain. It was possible to identify a statistical factor representative for Cd, Fe, Mn, Pb (Lorenzoni et al. 1997). We also carried out a geostatistical coregeonalization analysis and its results showed that correlation between Cu and other heavy metals is direct at small scale and inverse at large scale (De Simone et al. 1998). In all these cases the values of the investigated microelements indicated a status of non pollution of the Rieti plain soils. We know that in the last decades some chemical fertilizers, either enriched with micronutrients (e.g. Cu, Zn) or containing microelement impurities coming from production cycles, have been widely used in this area. Nevetherless it is not possible to quantify the total metal imput because it is very hard to obtain an exact report of the agricultural management (rotation of crops and chemical fertilizations) at each sampling sites. The aim of this research is to investigate the total and DTPA extractable contents of Cu, Mn, Pb and Zn by sampling in the same sites after 8 years, in order to monitor changes over the time due to the intensive agricultural practices and the proximity to the town of Rieti.

2. Materials and Methods

We collected 60 samples in a 90 km^2 area, according to a stratified random sampling scheme, from 0-40 cm plowed horizon (Ap). They include the main types of calcareous soils of the Rieti plain which were classified according to Soil Taxonomy (1992) subgroups of Typic Udifluvents, Fluventic, Fluvaquentic and Vertic Eutrochrepts, Aeric Haplaquepts and Typic Chromuderts.The samples were air dried, ground and sieved at 2 mm. Soil pH was measured by potentiometry both in H_2O and KCl 1N (1:2.5 soil-solution ratio), and total Organic Carbon was determined by the Walkley & Black method.Total metals were extracted by using 3 g of soil dissolved in 28 ml *aqua regia* (3 parts HCl and 1 part HNO_3) left two hours at 120 °C in Teflon bomb and diluted to 100 mL after cooling.

Easily extractable metals were extracted by 0.005 M DTPA solution, buffered at pH 7.3, which is widely used in near-neutral and calcareous soils (Lindsay and Norwell 1978).

All extracts were analyzed for metal concentrations by using a 1100 B Perkin Elmer flame Atomic Absorbtion Spectrometer.

The data have been analysed with ANOVA at $p = 0.05$ level using SPSS statistical software (SPSS inc.)

3. Results and Discussion

In Tab. 1 the main statistical parameters of the studied metals are displayed.

Results of Analysis of Variance regarding the comparison between the concentration of the same metal in the soils samples collected in two different years (1990 and 1998) show that no significant variations in both total and available fractions are present (Mn_{TOT} $F=0.16$, $p=0.69$;

Mn_{DTPA} 3.43, p=0.08; Cu_{TOT} F=1.23, p=0.28; Cu_{DTPA}F=0.14, p=0.71; Pb_{TOT} F=0.85 p=0.36; Pb_{DTPA}F=2.76 p=0.11; Zn_{TOT} F=0.39 p=0.53).

Table 1. Main statistical parameters of the studied metals.

variable	mean	variance	std. dev.	kurtosis	skewness	minimum	maximum
Cu DTPA 1990	5.6	17.38	4.17	4.04	1.89	0.8	25.0
Cu DTPA 1998	7.7	84.91	9.21	29.17	4.57	1.1	76.6
Cu total 1990	35.4	173.60	13.18	-1.31	0.72	22.0	56.0
Cu total 1998	47.3	523.35	22.88	2.70	1.52	14.9	127.5
Mn DTPA 1990	17.1	76.83	8.77	3.49	1.60	3.4	54.4
Mn DTPA 1998	29.4	221.94	14.90	4.20	1.45	3.9	98.9
Mn total 1990	633.9	6941.21	83.31	0.30	0.96	526.0	793.0
Mn total 1998	780.9	106085.14	325.71	2.43	1.40	88.3	1986.4
Pb DTPA 1990	3.1	14.81	3.85	72.64	7.83	0.4	41.9
Pb DTPA 1998	3.6	6.41	2.53	17.70	3.82	0.9	18.1
Pb total 1990	31.4	41.38	13.18	0.45	0.72	22.0	44.0
Pb total 1998	42.1	133.60	11.56	9.59	1.99	20.9	109.5
Zn DTPA 1990	3.7	0.09	0.30	2.10	6.60	0.6	13.6
Zn DTPA 1998	3.4	1.46	1.21	3.11	1.00	1.1	8.9
Zn total 1990	74.3	127.79	11.30	-0.66	0.95	63.0	95.0
Zn total 1998	79.9	322.48	18.23	-0.41	-0.19	29.5	114.6

4. Conclusions

The findings of this research suggest that the intensive agricultural practices in the Rieti plain area, as well as other anthropogenic activities, didnot result in a significant increase of the studied microelements content in soils over the considered period. Nevertheless the mean concentrations of the studied metals show a slightly increasing trend over the time though they still remain under threshold values fixed by the Italian regulations about agricultural soils.

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EFFECT OF COPPER SPRAY CHEMISTRY ON APPLE FRUIT RUSSETTING AND LEAF TISSUE COPPER STATUS

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1. Introduction

The concentration of copper (Cu) in apple trees has been declining in commercial orchards, likely because of reduced use of Cu-based pesticides and greater purity of fertilizer materials. Overt symptoms of Cu deficiency, expressed vegetatively as wither-tip and rough bark, have been reported with greater frequency. Analogy with non-fruit crops suggests that Cu deficiency may also influence fruit yield and quality. As a consequence, there is increasing use of Cu-containing foliar sprays applied expressly for nutritional purposes.

Some Cu-containing products are potentially phytotoxic, depending on the rate, timing, or method of application, or if they are applied in combination with or shortly before or after certain other spray products. Anecdotal examples include increased fruit marking when Cu sulfate is applied in acidified spray solutions, or when basic Cu sulfate is applied within a few weeks before or after calcium chloride sprays are made for control of the physiological disorder bitter pit. The current study was a preliminary attempt relate the chemistry of Cu in Cu nutritional sprays to an apple fruit finish problem (russetting) and leaf tissue Cu status.

2. Materials and Methods

The field portion of the experiment was conducted in a 'Golden Delicious'/M.9 apple orchard located at the Washington State University research farm in Wenatchee, Washington, USA. 'Golden Delicious' apple is a russet-prone cultivar. The free-standing trees are planted at a 6.25' X 15' spacing and are irrigated using a permanent undertree high pressure/high volume sprinkler system. The nine treatments were arranged in a randomized complete block experimental design using four blocks and four-tree plots.

The treatments were two mid-summer (13 June and 25 July 1997) foliar sprays of water, 0.84 L Copocal in combination with 0, 0.0037, 0.0372, or 0.3725 mol H_3PO_4/100 L spray (H_3PO_4 series), or 0.36 kg $CaCl_2$ in combination with 0, 0.28, 0.56, or 0.84 L Copocal/100 L ($CaCl_2$ series). All treatments were prepared using tap water and were applied using a backpack sprayer. The four trees within each plot were sprayed to drip. Tech-Flo Copocal is a proprietary nutrient spray product (Nutrient Technologies, La Habra, California, USA) formulated from basic copper sulfate [brochantite, $Cu_4SO_4(OH)_6(s)$] and dicalcium phosphate [monetite, $CaHPO_4(s)$] with a final composition of 5% Cu, 7.5% Ca, and 6.1% P (w/w) and a density of 1.366 kg/L. It is a viscous opaque blue-green liquid. We have found this product to be nonphytotoxic when applied alone in foliar sprays during the growing season.

The treatments were designed to provide one series of Cu suspensions with increasing concentrations of $Cu^{2+}(aq)$ free metal ion made by acidifying the Copocal solution with phosphoric acid, and a second series in which increasing amounts of total Cu were added relative to a fixed total chloride concentration, with the hope of forming differential concentrations of ion-pairs of Cu and Cl^-. Chemical speciation was carried out using the equilibrium speciation program MINTEQA2 (ALLISON et al. (1990)) coupled with the database of thermodynamic constants modified by W.L. Lindsay, Colorado State University,

Fort Collins, Colorado, USA The model inputs included measured solution chemical data and assumptions regarding which solid phases might be present and controlling dissolved ion activities. Two conceptual systems have been evaluated to date – one in which solution chemistry is controlled by metastable brochantite and monetite solid phases, and a second in which all known and relevant inorganic Cu and Ca minerals are allowed to precipitate if warranted by thermodynamic conditions.

3. Results and Discussion

The relative amount and severity of skin russetting on the apple fruit was unrelated to experimental treatment. The natural background incidence of severe russetting was high in the experimental block, averaging 18% in the fruit from the water-only treatments, which may have reduced the potential for inducing definitive treatment effects.

H_3PO_4 series - Leaf Cu concentration was weakly and inversely related to spray suspension pH, when total Cu concentration (aqueous plus solid phase) was held constant in all spray suspensions. The reduction in pH was accompanied by a corresponding increase in both total dissolved Cu and dissolved Cu occurring as Cu^{2+}(aq); however, the relationship between leaf Cu concentration and these factors was even weaker.

$CaCl_2$ series - Leaf Cu concentration was strongly and linearly related to the total Cu concentration (aqueous plus solid phase) in the spray suspension. The linearity of this relationship disappeared when leaf Cu concentration was correlated with either total dissolved Cu and dissolved Cu occurring as Cu^{2+}(aq) in the Copocal-containing treatments, because *de novo* formation of tenorite [CuO(s)] caused total dissolved Cu concentrations and dissolved Cu species distribution to be restricted to narrow ranges. The modeling results also suggested that the concentrations and proportions of ion-pairs formed from Cu and Cl^- remained relatively constant and therefore would not account for the observed differences in leaf Cu concentrations.

4. Conclusions

The results of this single-season field trial failed to show a definitive association between apple fruit russet and Cu nutrient spray chemistry. Copper concentrations in plant tissue appear to be poorly related to Cu speciation in the Cu nutrient spray suspension as initially prepared in the sprayer tank. The amount of Cu actually absorbed into plant tissue may be masked by Cu spray residues adhering to plant surfaces, and/or Cu phytoavailability from foliar sprays may be better modeled by incorporating the effects of evapoconcentration on solution composition and solid phase formation into the geochemical model.

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EFFECT OF SLOW RELEASE ZEOLITE-BOUND ZN AND CU FERTILIZERS ON CD CONTENT IN WHEAT

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1. Introduction

This work is part of a project concerning the effect of slow release zeolite-bound Zn and Cu fertilizers on micronutrient status and yield of crops. Due to antagonistic interaction of both essential micronutrients with Cd, the application of such fertilizers could also help to reduce the uptake of Cd by crops (Oliver et al., 1994; Grant et al., 1998). Plants can accumulate relatively high levels of Cd without adverse effects on growth, therefore consumption of foods high in Cd may induce chronic toxicity, because this element is retained for many years in the human body. Through the application of sewage sludge and phosphate fertilizers, the soil Cd content in many agricultural areas has been raised. Thus it is important to reduce the uptake of Cd so that critical levels in food are not exceeded. The aim of this work was to investigate the effect of application of Zn and Cu fertilizers, singly and in combination, on the Cd uptake of wheat.

2. Materials and Methods

Cadmiumsulphate was mixed with the experimental to raise the Cd level in soil to a content of 5.1 mg kg^{-1} and placed in 431 pots. Five months later the Cd-enriched soil has been mixed with different levels of Cu- and Zn-zeolites (Table 1),with each treatment being replicated 3 times. Then the pots were exposed to free air, and planted with spring wheat cv. Nandu.

Table 1: Amounts of zeolite bound Zn and Cu applied

Variety	applied Zn	applied zeolite	Zn- corresponds to kg Zn	applied Cu	applied zeolite	Cu- corresponds to kg Cu
control	0	0	0	0	0	0
Zn 1	0.33	4.78	20	0	0	0
Zn 2	0.83	12.03	50	0	0	0
Cu 1	0	0	0	0.167	3.77	10
Cu 2	0	0	0	0.501	11.33	30
ZnCu 1	0.33	4.78	20	0.167	3.77	10
ZnCu 2	0.83	12.03	50	0.501	11.33	30

Plant material was oven-dried at 105 °C and digested by a mixture of nitric and perchloric acid (5:1 v/v). The soil samples were air-dried, passed through a 2 mm sieve and extracted by aqua regia under reflux. The element contents were determined by ICP - AES (Varian Saturn).

3. Results and Discussion

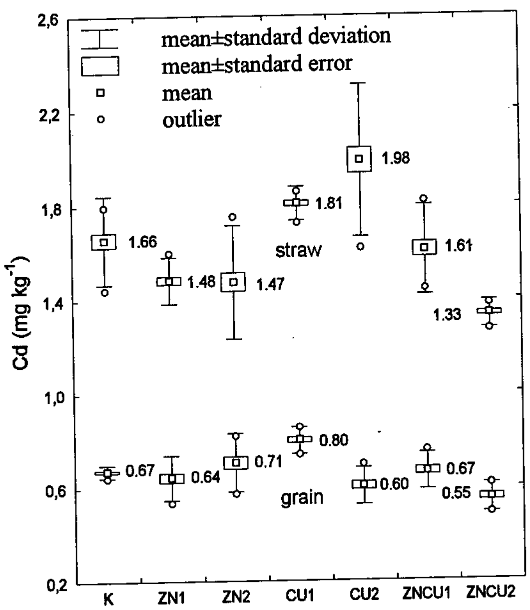

Figure 1: Cd content in straw and grain

Figure 1 shows the Cd contents of straw and grain. It can be seen, that there is a reduction of about - 10 % Cd content in straw when Zn is applied alone, however, the Cd levels in grain did not change. Cu fertilization lead to higher Cd levels in straw (+ 19.9 % for Cu2); in grain Cu1 showed an increase of Cd levels (+ 18.9 %), but for Cu2 the grain Cd content has decreased compared to the control (- 10.1 %). However, both straw and grain Cd levels decreased most when Cu and Zn were applied together, the ZnCu2 treatment shows a decrease of Cd level of about - 20 %.

Although rather high amounts of Zn and Cu were applied, the effect on Cd uptake was relatively low, especially the addition of Zn alone which only led to a decrease of Cd content in straw but not in grain. Only the application of Zn and Cu together had significant effect on Cd levels in straw and grain, respectively. Possible reasons are, that the tested soil was not deficient in Zn and Cu and that only a small amount of the applied micronutrients has been released to soil solution. Low increase in plant availability has little effect on antagonisms and thus little effect on Cd availabiltiy. Therfore, further work is required to fully assess the effectiveness of zeolite fertilizers to decrease the Cd concentration of wheat grain.

4. Conclusions

This study has shown that grain Cd content can be decreased most effectively, when zeolite-bound Zn- and Cu-fertilizers are applied together. In this case the wheat grain Cd concentration can be decreased by up to -20 % by the addition of 50 kg ha^{-1} Zn and 30 kg ha^{-1} Cu. Application of Zn-zeolite alone only had influence on Cd levels in straw but not in grain.

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STATUS OF SOME MICRO-NUTRIENTS ON SOILS OF MADHUPUR TRACT IN BANGLADESH

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1. Introduction

Soil has to furnish adequate amounts of essential nutrient elements for plant growth. The total amount of essential nutrient elements in a soil does not give an indication of nutrient supplying power of the soil. Only a fraction of the total supply is accessible to plants in available form. When the soil does not furnish adequate quantities of any nutrient element then plants suffer from its deficiency. An adequate supply of the element is to be added in available form.

With the objective in mind the present piece of work was initiated to study the status of four trace elements, in the different horizon of two soil of Madhupur Tract. Several chemical extractants to evaluate trace element status of the soils were used and their status evaluated against critical levels for satisfactory plant growth.

2. Materials and Methods

The Madhupur Tract soil of Gazipur District was selected for the study (SRDI, 1973). The pH, organic carbon, clay content of air-dried soils water suspension was determined using standard procedures. Total amount of trace elements in soils was determined after digestion with perchloric-nitric acid mixture.

The *1N* NH_4OAC (pH 7.0) (Page et. al, 1985), *0.1N* HCl (Nelson et al.,1959), and water extraction methods were employed for extracting exchangeable elements Fe, Mn, Cu and Zn, followed by their determination by atomic absorption spectrometry.

3. Results and Discussion

Soil pH varies from 4.3 to 6.4 generally increasing with depth. The root zone pH was below 5.0 in all soils. Organic carbon content of all soils was very low. The soils contain high amounts of clay fraction.

Results show that the total amount of iron, manganese, copper and zinc are high in the soils under investigation. Their availability indices are quite low. However, all elements were in adequate available form. Critical levels of these elements as reported by Bangladesh Agricultural Research Council (1997) are much below those found in these soils. Low pH

influenced the availablity of iron and manganese. At the surface horizon of the soils, _1N_ NH_4OAC (pH 7.0) extractable elements were 34 to 36 μg g^{-1} Fe, 23 to 26 μg g^{-1} Mn, 1.5 to 4.3 μg g^{-1} Cu, and 5 to 6 μg g^{-1} Zn. Maximum variation was in the case of copper where Gerua soil had, on average, 1.7 μg g^{-1} and Kalma soil had 4.2 μg g^{-1}.

4. Conclusions

For plant growth, measurement of available nutrient content in soil is more important than their total content. Environmental factors, such as soil moisture and temperature conditions, may affect the availability of micronutrients in soil particularly when oxidizing condition prevails and high temperature accelerates the reaction.

Positive correlation between Mn, Cu, and Zn with organic matter was found. Iron had a negative correlation probably because anaerobic condition of soil which keeps Fe available to plants does not help in organic matter buildup.

Table 1. Content of iron, manganese, copper, and zinc in some soils of Madhupur Tract, Bangladesh (in ppm).

Soil	Depth	Iron				Mnaganese				Copper				Zinc			
	cm	Total	HCl	NH4O Ac	Water	Total	HCl	NH4O Ac	Water	Total	HCl	NH4O Ac	Water	Total	HCl	NH4O Ac	Water
Gerua 1	0-38	747	541	35.25	0.00	44.55	26.55	23.45	2.45	23.45	1.75	1.54	1.22	16.65	9.40	6.33	2.12
	38-89	835	573	35.45	0.00	44.25	18.40	18.25	1.79	24.50	1.60	1.25	1.11	17.55	8.55	6.25	2.11
	89-127	797	574	35.68	0.00	38.45	18.30	17.50	1.65	25.35	1.65	1.12	1.12	19.70	11.15	6.25	2.09
	127-229	1008	574	36.12	0.00	38.15	16.30	15.35	1.55	24.40	1.55	1.27	1.14	26.60	9.15	6.22	2.06
	229+	1082	632	38.55	0.00	35.40	16.45	10.30	1.25	25.35	1.75	1.56	1.12	31.10	9.60	5.88	1.85
Gerua 2	0-18	763	592	36.25	0.00	41.25	29.75	23.40	1.55	24.50	2.50	1.75	1.34	14.65	9.05	5.86	1.89
	18-76	814	583	38.12	0.00	42.55	16.60	13.34	1.34	26.35	2.40	1.66	1.25	16.50	9.45	5.82	1.68
	76-119	854	573	38.64	0.00	46.15	16.35	11.80	1.56	23.30	2.25	1.64	1.32	18.60	11.15	5.67	1.66
	119-157	1094	505	38.41	0.00	35.25	16.35	12.50	1.34	22.20	1.80	1.54	1.32	18.50	13.10	6.24	1.89
	157-246	1177	584	38.25	0.00	34.40	19.35	18.10	1.65	19.40	2.05	1.44	1.25	21.70	9.70	6.12	1.88
	246+	1097	618	38.95	0.00	33.15	18.50	12.60	1.14	18.30	2.45	2.15	1.12	34.85	11.60	6.10	2.11
Kalma 1	0-18	681	542	32.56	0.00	33.30	28.50	25.95	2.75	22.35	6.40	4.25	1.56	12.50	7.74	5.11	1.12
	18-36	693	521	34.45	0.00	28.70	25.35	19.60	2.15	20.60	6.05	4.32	1.66	15.30	8.86	5.09	1.11
	36-81	691	583	34.48	0.00	34.50	28.40	21.35	2.45	19.40	3.55	2.55	1.32	13.30	8.82	4.95	1.09
	81-152	581	452	33.47	0.00	27.65	15.55	14.75	1.75	16.35	2.50	5.12	1.76	15.40	8.60	4.96	1.05
	152-203	574	424	33.71	0.00	26.50	17.25	17.50	1.38	15.35	2.30	2.11	1.42	18.70	11.45	5.85	1.08
	203+	562	405	33.49	0.00	24.25	16.45	10.15	1.15	15.80	2.25	1.98	1.24	23.70	11.65	5.86	1.10
Kalma 2	0-18	677	521	34.58	0.00	53.40	33.65	12.50	2.56	23.50	7.20	3.86	1.75	14.60	6.60	4.56	1.15
	18-28	634	512	35.45	0.00	44.65	31.35	11.30	2.45	19.85	6.60	4.12	1.67	15.60	8.15	4.85	1.06
	28-61	665	568	36.45	0.00	41.30	28.45	18.40	1.28	18.60	3.55	2.12	1.12	16.75	9.55	5.11	1.07
	61-119	505	443	34.25	0.00	41.25	16.60	10.90	1.14	19.35	3.45	2.05	1.25	25.65	11.60	5.86	1.11
	119-183	505	411	33.25	0.00	35.45	16.35	16.75	1.45	15.50	2.45	1.85	1.11	26.80	12.15	6.25	2.08
	183+	505	409	33.32	0.00	35.45	16.35	17.50	1.66	14.40	2.50	1.75	1.12	28.80	12.10	6.12	2.06

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EFFECT OF FUNGICIDE SPRAYS ON THE COPPER CONTENT IN SOILS

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1.Introduction

Addition of compounds to soils and crops has become a common practice in agriculture for the crop protection and disease control. These practices may cause chemical degradation of soils, as the result of compounds at undesirable levels.

Hence long term and the intensive horticultural use with the frequent application of fungicide sprays can cause detrimental effect to the environment due to accumulation of copper in the soil. Its interference with other ions for binding sites of soil and plant roots also cannot be ruled out because of similar ionic radii.

The copper added to the soil, persists as it seems to be strongly fixed by organic matter, clay minerals and hydrated oxide of Fe, Al and Mn (Schnitzer, 1969). The elevated concentration of copper in the surface soils due to copper fungicide spraying has been reviewed earlier (Tiller and Merry, 1981; Baker, 1990). Copper is seen to accumulate near the surface of the soil and hence, and root system of horticultural crops are affected. The aim of our work was to evaluate the effect of long-term cultivation with application of copper sprays on soil from Bahía Blanca horticultural belt.

2. Materials and Methods

Samples were selected in such a manner that they are representative of the age and persistence in the use of the sanitary products. Simultaneously samples were collected from similar depths from adjoining non-treated with fungicide sprays (Control: C). The study area was divided up into three sectors (S.1, S.2 and S.3). Each one comprises varying number of farms (F) (4, 1 and 2 respectively), which in turn are composed of several allotments with different crops (11, 5 and 28 respectively).

The samples from each allotment were taken form various depths (0-15, 15-30, 40-45 and 45-60 cm) and which have been characterised, by pH and clay and organic matter content.

The total (Marcet al., 1997) and DTPA extractable Cu (Lindsay and Norvell, 1978) were determined using atomic absorption spectrophotometry.

3. Results and Discussion

Results are in table 1. In the soils treated with fungicide sprays, the DTPA–extractable copper (Cu_e) ranged from 19 to 165 $mg \cdot kg^{-1}$ for the 0-15 cm depth, and from 18 to 35 $mg \cdot kg^{-1}$ for the 45-60 cm depth. Fewer differences have been found for total Cu contents.

4. Conclusions

Cu accumulates in surface layers, and the highest amounts occur in soils that have been longer treated with sprays. Clays and organic matter contents notably contribute to Cu retention; consequently, differences due to the persistence in the use of pesticides are attenuated. Cu fixes in the surface layer the more when the bigger organic matter content, showing little mobility to subsurface zones. The control treatment shows the lowest concentrations, thus confirming the

anthropic origin of Cu contents in the other soils.

Table 1. Depth variation of pH, O.M.and Cu content in the soils.

Sector/Farm	Depth (cm)	pH	Clay (%)	M.O. (%)	Cu_e (mg.kg^{-1})	Cu_t (mg.kg^{-1})
S1. F1	0-15	8.50	12.50	2.78	74	186
S1. F1	15-30	8.60	17.40	2.21	39	70
S1. F1	30-45	8.60	10.40	1.52	22	50
S1. F1	45-60	8.90	16.30	2.11	35	50
S1. F2	0-15	8.50	35.60	4.81	165	360
S1. F2	15-30	8.90	32.10	3.08	82	143
S1. F2	30-45	8.90	27.90	2.24	41	63
S1. F2	45-60	8.90	27.90	2.12	18	60
S1. F3	0-15	8.50	35.60	4.81	123	286
S1. F3	15-30	8.90	32.10	3.08	72	148
S1. F3	30-45	8.90	27.90	2.24	32	56
S1. F3	45-60	8.90	27.90	2.12	18	56
S1. F4	0-15	8.50	12.40	2.78	74	158
S1. F4	15-30	8.60	17.40	2.21	32	65
S1. F4	30-45	8.60	10.40	1.52	18	52
S1. F4	45-60	8.90	14.30	2.11	25	52
S2. F5	0-15	8.70	20.59	2.10	33	70
S2. F5	15-30	8.80	29.00	1.90	24	62
S2. F5	30-45	8.70	20.59	2.10	18	53
S2. F5	45-60	8.70	20.59	2.10	18	50
S3.F5	0-10	8.50	17.50	1.54	19	50
S3. F6	10-20	7.90	17.50	1.95	19	48
S3. F6	20-30	8.50	17.60	1.72	23	66
S3. F6	30-40	8.70	20.10	1.13	23	60
S3. F6	40-50	8.70	20.10	0.90	23	40
S3. F6	50-60	8.70	20.10	0.90	24	40
S3. F6	60-80	8.70	20.10	0.56	25	40
S3. F7	0-10	8.10	27.40	3.11	12	46
S3. F7	10-20	7.80	34.90	3.81	25	38
S3. F7	20-30	7.80	22.50	1.04	25	38
S3. F7	30-40	7.60	29.80	1.56	31	38
S3. F7	40-60	7.70	27.40	0.92	23	38
S3. F8	0-15	7.80	18.60	1,47	56	145
S3. F8	15-30	7,60	17.20	0.86	37	75
S3. F8	30-45	7.80	17.50	0.75	28	46
S3. F8	45-60	7.80	17.50	0.55	22	46
FC	0-20	7.80	8.50	4.81	8	26
FC	15-30	7.60	7.20	3.08	5	18
FC	30-45	7.80	7.50	2.24	u.l.	6
FC	45-60	7.80	7.50	2.12	u.l.	6

u.l.: undetectable level

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EFFECT OF IRRIGATION SCHEDULING ON CADMIUM UPTAKE BY LETTUCE

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1. Introduction

One of South Australia's most diverse horticultural areas is located nearby the major sewage treatment plant (STP) of Adelaide (South Australia's capital). Soon water recycled (tertiary treated and disinfected) from the STP will be available for unrestricted irrigation of a range of horticultural crops. The recycled water contains low levels of trace metals, including cadmium (Cd). However, Cd has been applied to soils in the area as impurities in phosphatic fertiliser and could become more available for crop uptake through irrigation with the recycled water, which is generally more saline than the bore water in this area.

Soil and water conditions that promote uptake of Cd by plants are; high Cd concentrations, high salinity (McLaughlin *et al.* 1994), low pH, low soil organic matter and low soil Zn concentrations (McLaughlin *et al.* 1996). Cadmium concentrations in crops grown under a combination of these conditions have exceeded maximum permissible concentrations (MPC) (McLaughlin *et al.* 1996). Irrigation scheduling may also influence availability of Cd by modifying soil solution Cl and Cd concentrations in the root zone. The aim of this work was to determine if Cd uptake by lettuce could be affected by irrigation scheduling and water source.

2. Materials and Methods

Single plants of lettuce (*Lactuca sativa* cv. Iceberg) were grown for 112 days in pots containing 12 kg of soil in a glasshouse (temperature range 16 – 26°C). The experiment was arranged in a randomised block design with three soils (one only presented in this paper), two water treatments (recycled water (RW) and bore water (BW)) and three irrigation scheduling treatments. Selected soil and irrigation water properties are listed in Table 1.

Table 1. Initial soil and irrigation water properties

Soil		Irrigation water		
Property	Value	Property	Bore Water	Reclaimed water
$pH_{1:5\ water}$	8.5	pH	7.5	8.1
$EC^{A}_{1:5\ water}$ (mS cm^{-1})	0.13	EC^{A} (mS cm^{-1})	1.1	1.8
Cl (mg kg^{-1})	20	Cl (mg L^{-1})	230	410
EDTA-extractable Cd (mg kg^{-1})	0.09	SAR^{B}	3.5	8.7
Organic Carbon (%)	0.5			

[A]Electrical conductivity; [B]Sodium adsorption ratio

Irrigation scheduling was defined as: minimal (soil water potential) -0.8 to -0.18 bar; optimal -0.18 to -0.07 bar; and leaching < -0.04 bar with a 20% leaching fraction. When soil water for two or more pots per treatment reached predefined lower potentials, appropriate volumes of water were applied to increase potentials to the upper predefined value for that treatment.

3. Results and Discussion There was no relationship between lettuce dry weights and Cd concentration in lettuce (Figure 1) and therefore no effect of growth dilution on Cd concentrations. Irrigation scheduling significantly affected plant Cd concentrations - at low mean soil water potentials concentrations of Cd in lettuce were significantly greater (and equalled the MPC) than at high mean potentials for both irrigation waters (bore water $P < 0.05$, recycled water $P < 0.001$). The mechanism for increased Cd uptake by lettuce was likely due to increased soil solution Cl concentrations induced by the different irrigation scheduling regimes (McLaughlin *et al.* 1994). The irrigation scheduling which included leaching prevented soil solution Cl concentrations from increasing, while the treatment having low mean soil water potentials allowed soil solution Cl and Cd concentrations to increase (data not shown). For each irrigation treatment there were no significant ($p<0.05$) differences between water types (Figure 1). These data suggest that although Cl loading in reclaimed water was approximately double (Table 1) that of bore water, irrigation scheduling overode any effects due to water quality.

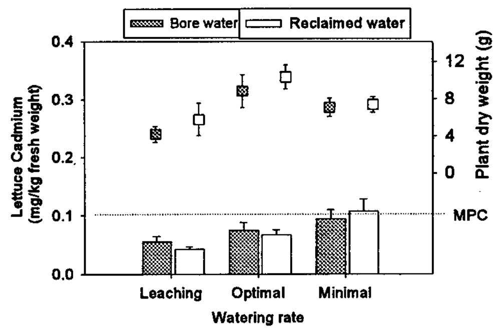

Figure 1. Effect of irrigation scheduling on yield and uptake of cadmium by lettuce. Error bars = one standard deviation from the mean, n = 4. MPC = maximal permissible concentration.

4.Conclusions

These data suggest that irrigation management could be used by growers to overcome potential problems with Cd concentrations in produce. However, with recycled saline water, there may be a conflict between developing irrigation scheduling to reduce drainage of excess saline water through the profile, and irrigation scheduling designed to minimise Cd accumulation in crops. Further work is required to test this hypothesis in the field.

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TRACE METALS IN SOILS OF AGRICULTURAL LAND OF POLAND

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1. Introduction

Soil quality is highly depended on concentrations of trace metals in the soil surface layers. Topical area in the land use program is the classification of trace metal status of soil, as one of basic criteria for the agricultural recommendations. The classification of soils according to levels of selected metals (Cd, Cu, Zn, Pb, and Ni), presented on maps, has became a fundamental information for the environmental and farming actions in Poland.

2. Materials and Methods

The collective soil samples from the surface layer (0-20 cm depth) were taken up in 32,167 sites located within the whole territory of Poland, in the period 1991-1995. Total contents of trace metals were measured in air-dried soil samples, after digestion in concentrated HCl/HNO_3 acids (aqua regia), by AAS spectroscopy. Analytical errors estimated using reference material and multiplied analyses varied from 5 to 20%.

3. Results and Discussion

Geometric mean contents of over 32,000 soil samples (Table 1) are relatively low and comparable to those in soils of the World (Kabata-Pendias and Pendias 1992), and could serve as the contemporary background levels. These values, however, differ significantly within textural soil groups. Therefore it is advisable to related metal background values to other soil properties, such as clay fraction content, pH etc.

Table 1. Trace metals (mg kg $^{-1}$ air dried) in soils of Poland and classes of metal status

Metal	Range	Mean	Classes of metal status - relative proportion (%)					
			0	I	II	III	IV	V
Cd	0.01 - 49.73	0.22	88.6	9.8	1.1	0.3	0.1	0.1
Cu	0.2 - 725	12	96.5	3.1	0.2	0.1	0.1	0
Zn	0.5 - 2838	40	87.9	10.7	1.2	0.2	0	0
Pb	0.1 - 5000	15	97.0	2.5	0.3	0.2	0	0
Ni	0.1 - 328	25	94.8	4.7	0.4	0.1	0	0

The target values for trace metals are established basing on the ecological risk assessment and safety factor for metal exposure of man due to consumption of plant food (Kabata-Pendias et al. 1993.) The concentrations of Cu, Pb and Ni in over 90% of soils are at the natural levels, while Cd and Zn is not changed only in about 87% of soils (Table 1). When the classification of soils is related to a given target values for each soil textural group and for variable pH, the assessment of soil metal levels is different (Table 2). Only 78% of soils reveal the sum of metals (Cd, Cu, Zn, Pb, Ni) at the 0 class level. Both soil classes 0 and I accepted as suitable for all crop plants (including vegetables for infants and children) account for 97% of the rural land. It means that 3% soils have elevated concentrations of metals, and only 0.1% is recommended for the exclusion from farming. On 0.3% land cultivation of industrial plants would be preferable.

Table 2. Synthetic soil classification, based on the sum of trace metals, and related to soil properties (relative proportion in soil classes, %)

Soil classes of metal status - relative proportion (%)							
0	I	II	III	IV	V	0 + I	II - V
78.4	18.6	2.2	0.4	0.3	0.1	97	3.0

4. Conclusions

A general classification of soils of Poland indicates that "pristine" levels of trace metals have dominated in the agricultural land. Almost all soils classified within III and V groups are located in the south western part of Poland, e.g. Upper Silesia (Terelak et al. 1997). Some soils contain elevated amounts of metals, up to the class III, on very limited areas of Carpatian Piedmont, and in the northern belt of loamy soils derived from glacial till. Trace metals in soils of those regions are considered as lithogenic due to the evidence of their inheritance from the lithosphere (parent material).

Elevated Cd levels in certain regions of Poland, and especially in Upper Silesia are of a real agricultural concern. This is the most easily available metal to crop plants, and one of the greatest risk to man health. Thus, the project will be curried out for detailed monitoring of Cd pollution of soils and for special guidelines for farming in those areas.

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Trace Element Interactions with Humans and Animals

(Technical Session 3)

INFLUENCE OF SODIUM AND BARIUM SELENITE ON SELENIUM CONTENT IN SOME ORGANS IN SHEEP

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1. Introduction

The problem of deficiency of selenium as a trace element indispensable for humans and animals is widely known because hyposelenosis may cause numerous diseases (Arthur et al, 1994; Wolfram, 1992; Chanoine et al, 1993). Sheep is the most sensitive animal to the lack of selenium and may be used as „indicator animal" in endemic areas. The purpose of this study was to compare assimilability of selenium and its duration in the body on the basis of its content in blood serum and in selected tissues in sheep, following administration of sodium selenite (Na_2SeO_3) and barium selenite ($BaSeO_3$).

2. Materials and Methods

The study was performed on 18 sheep, subdivided into 3 equal groups. Group I received feed supplemented with 0.3 ppm sodium selenite, group II sheep were fed 0.3 ppm barium selenite supplemented feed and the remaining animals (group III) served as controls. In order to determine selenium content in the serum, blood samples were collected before the experiment and in its 2nd, 6th, 10th, 14th, 18th and 26th week. The administration of both a.m. compounds was discontinued after 14 weeks of the experiment. Then 3 sheep of each group were sacrificed and their tissues sampled. Selenium concentration in serum and tissues was determined by the method of absorption spectrophotometry (AAS) with hydride generation.

3. Results and Discussion

Oral administration of sodium selenite and barium selenite in sheep was found to produce a highly significant ($P<0.001$) increase in selenium concentration in the serum (Table 1) in both experimental groups, 10 times greater than in the control group (0.35 ± 0.10 and 0.36 ± 0.08 µmol/l.). Despite considerable diminution of selenium level after its application had been interrupted, its concentration in groups I and II were still significantly higher ($P<0.01$) in the 26th week. Similar findings have been recorded by Judson et al. (1991) and Sankari et al. (1983).

Another effect of oral administration of sodium selenite and barium selenite consisted in selenium growth in the tissues (Table 2). The selenium concentrations in the liver, kidneys and heart were found to be greater in group I than in group II, similar in both groups in case of the muscles and higher in group II than in group I for Se level in the spleen. In all sheep receiving selenium, its concentration in the liver and kidneys was found to be 10-15 superior to its concentration in the heart, spleen and muscles. In the control group, on the other hand, the highest selenium concentration was recorded in the kidneys, followed by that in the heart, spleen and muscles and the lowest one in the liver. Zachara et al. (1993) and Echevarria et al. (1988) also demonstrated a marked growth of selenium concentration in various tissues but their results had a high degree of variance. This is due to the fact that such experiments give different results depending not only on

the strength of the applied selenium dose but also on the form of its compound and the route of administration.

Table 1. Selenium content (mean values ± standard deviation) in the sheep blood serum in μmol/l

Group	Weeks						
	0	2	6	10	14	18	27
I	0.34±0.19	4.08±0.75	4.09±0.84	4.10±0.54	4.38±0.56	1.60±0.12	0.84±0.18
II	0.33±0.10	2.90±1.03	4.55±0.21	3.79±0.62	3.97±0.77	2.35±0.31	0.96±0.13
III	0.34±0.08	0.35±0.13	0.36±0,10	0.36±0.08	0.35±0.10	0.35±0.13	0.41±0.13

Differences between groups I and II and group III were, in all time intervals (except "0"), statistically significant at $p<0.001$ (in the 27th week: at $p<0.05$ for group I and $p< 0.01$)

Table 2. Mean selenium concentration in the examined tissues in the sheep (μg/g net weight)

Group	Tissues				
	muscles	spleen	heart	kidneys	liver
I	0.35 (0.27-0.49)	0.43 (0.30-0.56)	0.63 (0.30-0.84)	3.19 (2.50-4.46)	5.33 (3.95-6.76)
II	0.31 (0.11-0.47)	0.64 (0.44-0.75)	0.26 (0.19-0.32)	2.38 (2.00-2.65)	4.15 (3.50-4.65)
III	0.10 (0.08-0.12)	0.16 (0.10-0.22)	0.16 (0.15-0.20)	0.62 (0.18-1.33)	0.03 (0.02-0.05)

4. Conclusions

The obtained results indicate that increased concentration of selenium in the blood serum was more persistent in sheep treated with barium selenite. Of the tissues tested, the highest concentration of selenium was found in the kidneys and liver. In contrast, the muscles revealed the lowest amount of selenium. Adequate selenium supplementation of feed, applied in animal breeding in endemic areas, may serve as an indirect preventive measure in selenium deficiency cases in humans.

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ANTAGONISTIC RELATIONSHIPS BETWEEN SELENIUM AND MAGNESIUM IN SHEEP

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1. Introduction

Magnesium and selenium are essential elements for man and animals. Their physiological role is strictly linked to the interactions, and particulary to the antagonistic influence of other elements (Kabata-Pendias and Pendias 1993, Anke et al. 1994). It has been demonstrated that an increase in selenium tissue level results, among other, in a corresponding magnesium decrease in the tissues (Kabata-Pendias and Pendias 1993, Zhu et al. 1993). The aim of the present study was to evaluate the effect of sodium selenite administered parenterally on magnesium concentration in the serum of sheep.

2. Materials and Methods

The experiments were carried out 12 sheep, divided into two equal groups. Group I consisted of sheep injected twice with 5 ml of 0.22% aqueous solution of sodium selenite (Na_2SeO_3), equivalent to 5 mg selenium. Group II served as the non-treated control. Blood samples from sheep were collected 1 month before and on the day of selenium injection, and then twice at 1 month interval after selenium injection. The concentration of selenium and magnesium in blood serum was determined by means of the atom absorption spectrometry (AAS, Perkin-Elmer, Model 3110). The serum samples for selenium determination were mineralised according to the Welz and Melcher metod (1984) and analysed in the spectrometer equipped with an attachment for hydrides generating (MHS 10). The results obtained are illustrated as arithmetic means and were analysed statistically using Student's t-test at $P < 0.05$.

3. Results and Discussion

Selenium concentration in the serum of sheep of Group I increased markedly from the initial value of 0.42 ± 0.11µmol/l up to 1.38 ± 0.21 in the first month and up to 1.10 ± 0.20 µmol/l in the second month after the sodium selenite injection, as compared to the values found in Group II (Table 1). More than a three-fold increase in selenium concentration observed in Group I of sheep a month after the first administration of sodium selenite, was statistically significant as compared to the mean control value (0.41 ± 0.10 µmol/l). Although in the second and third months of the experiment selenium levels were significantly higher in sheep of Group I than in Group II, a decreasing tendency of the selenium levels was evident. Similar changes in selenium concentration in sheep serum after administration of various selenium compounds have been reported by other authors (Judson et al. 1991, Zachara et al. 1993). Judson et al. (1991) have also found that the marginal selenium concentration in sheep serum should range from 0.25 µmol/l to 0.50 µmol/l.Sodium selenite administration in sheep resulted in a marked diminution of magnesium concentration in the serum, from the initial level of 1.05 ± 0.05 to 0.88 ± 0.03 in the first month, and to 0.82 ± 0.07 mmol/l in the second month of the experiment. In Group I of sheep, magnesium

concentration in the first and second month of the experiment was significantly lower than in sheep from Group II. This drop in magnesium concentration in the serum of sheep after sodium selenite injections may indicate antagonistic relationships between selenium and magnesium. This hypothesis is in accordance with the concepts of several researchers (Kabata-Pendias and Pendias 1993, Anke et al. 1993). Zhu et al. (1993) examined the effect of magnesium deficiency on the distribution of selenium in rats and found that selenium concentration rose in the muscles and dropped in other tissues. However, Gosh et al. (1993) have demonstrated alleviated symptoms of selenium poisoning in buffaloes grazing in the Himalaya Mountains and exposed to magnesium.

4. Conclusions

Our results confirmed an antagonistic action of selenium and magnesium described by several researches. However, this relationship is complex and needs more detailed studies.

Table 1. Selenium and magnesium contents (mean ± standard deviation) in serum of sheep

Parameter	Group	Months			
		0 - ↓	1 - ↓	2	3
Selenium (µmol/l)	I	0.42 ± 0.11	1.38 ± 0,21*	1.10 ± 0.20*	0.63 ± 0.14*
	II	0.43 ± 0.08	0.41 ± 0.10	0.38 ± 0.10	0.35 ± 0.08
Magnesium (mmol/l)	I	1.05 ± 0.05	0.88 ± 0.03*	0.82 ± 0.07	0.87 ± 0.14
	II	1.03 ± 0.08	1.00 ± 0.11	0.97 ± 0.09	0.97 ± 0.09

Group I - Na_2SeO_3; Group II - control; ↓ - injections of Na_2SeO_3

* - differences statistically significant at P<0.05, inthe adequate time intervals

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BIOAVAILABILITY OF PARTICULATE METALS TO FISH GILLS

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1. Introduction

Much of the recent literature in environmental biogeochemistry indicates that the speciation of trace metals in a natural water system is more meaningful than its total concentration (1). Therefore, chemical speciation in an aquatic system is of central importance to bioavailability. Although the concepts of bioavailability and speciation have been widely accepted, the processes governing the bioavailability of metals to aquatic organisms are still poorly understood. The fundamental chemical and biological processes active in aquatic systems, the uptake pathway and mechanisms of various metal species in particular, are critical to a better understanding of metal bioavailability and toxicity. The gills, as the primary uptake site in fish for trace metals, are the most important targets when exposed to elevated ambient metals. It is generally recognized that the hydrate ions and hydroxo complexes are the most, or sole, bioavailable forms of metals for fish uptake via the gills among all of the species which exist in the aquatic environment (2). Suspended particles in aquatic environment often act as competitors for free metal binding and suppress bioavailability of metals. However, there is no sound experimental evidence on bioavailability of these species as far as fish uptake via gills is concerned. The objective of this study was to investigate the bioavailability of particulate lead to fish via uptake by the gills.

2. Materials and Methods

Carassius auratus (size ca.120 mm) was exposed to suspended particulates (gibbsite) adsorbed lead. Four fish were transferred to each glass tank holding 1000ml of synthetic water spiked with lead and gibbsite. The particulate lead was controlled at varied concentrations (0, 0.4, 0.8, 1.2, 2.0, and 3.0 mg/l) while a constant low level of free lead (0.1mg/l) was maintained. Lead and aluminum in the gills and intestines of the fish were determined after the exposure. The gills were also examined with a light microscope for any particles adhering to the surface. Fish gills were digested and determined for lead after the exposure using a PAR polarograph, model 303 in a differential pulse anodic stripping mode.

3. Results and Discussion

The fish were exposed to particulate lead at concentrations ranging from 0 to 3.0 mg/l (with free lead of 0.10 mg/l). The resulting measured lead contents of the gills after the exposure treatments are shown in Figure 1. The accumulation of lead in the gills demonstrated an elevated accumulation trend. For the fish exposed to solely free lead at concentrations of 0.10mg/l, lead accumulation in the gills during five-day exposure was estimated to be 3.1 mg/kg which was much lower than those shown in Figure 1 (4.07 to 56.5 mg/kg). Lead accumulation increased almost linearly with increases in particulate lead concentration. Such results indicate that particulate lead was available to fish not only through ingestion, but also via the gill respiration process. As with accumulations of lead, aluminum accumulations in the gills increased with increases in the gibbsite contents of the water. Even though gibbsite

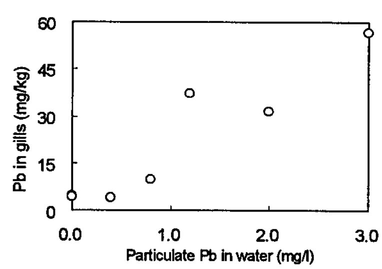

Figure 1. Lead in the gills of the fish exposed to various levels of particulate lea (dissolved lead 0.10mg/l)

is generally insoluble in water under experimental conditions, some dissolution of trace amounts of aluminum from the particles could not be entirely counted out. It can be calculated that the concentration ratio in the suspended gibbsite with lead adsorbed was close to 100 (mg/kg over mg/kg) in the ambient water. However, the calculated ratios of aluminum and lead in the gills varied from 1.25 to 10.10. Even when the contribution of free lead (0.1 mg/l exposure rate) was taken into consideration, the ratios of aluminum and lead in the gills would not exceed 20. The more favorable lead accumulation in the gills when compared with aluminum suggest some mechanism may be at work thus accounting for the possible lead stripping by the fish.

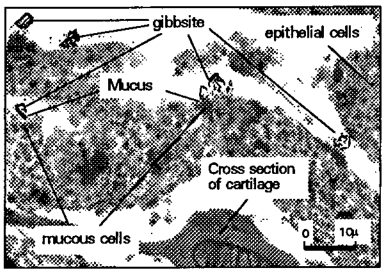

Figure 2 Gibbsite particles attached to the surface of the gill epithelial cells (light microscopy 400 X)

It was suggested that during aspiration, the particles came in contact with and adhered to the surface of the gills which were covered by a layer of mucus. To confirm this speculative assumption, photos were taken of cutting sections of the gills with one example shown in Figure 2. The process of gill section cutting involves a series of solution soaking and washing procedures during which some of the particles adhering to the gills might be washed away along with some mucus. Still, many gibbsite particles were observed adhering to the surface of the gill epithelia where some mucus remained. The gibbsite particles were identified by comparing photos of the cutting section with those of the suspended solution of pure gibbsite particles serving as a control. Although the maximum size of the particles was 10 μm in the solution, the size (longest axis) of most particles or particle aggregates observed in the more than 20 of the photos taken was less than 4μm.

All of the evidence presented appears to confirm the belief that particulate lead was available for fish bioaccumulation via the gills. Martens and Servizi studied suspended sediment particles inside the gills of *Oncorhynchus spp.* They found that the particles could be phagocytosed by gill epithelial cells (3). In this study, however, no gibbsite particle was observed within the epithelial cells. The relatively low ratio of aluminum to lead in the gills also implied the possibility of some lead stripping process. The stripping, or desorption, of lead from the gibbsite particles could occur within the microenvironment which is different from the bulk solution (4). It is thus proposed that the translocation of the lead adsorbed on to the gibbsite particles underwent three sequential steps: 1) The particles adhered on the mucus of the epithelial cell surface; 2) the lead adsorbed on the particles desorbed under conditions of the gill microenvironment; and 3) the released lead translocated into the gill tissue. The entire process of the particulate lead uptake by the gills is illustrated as a conceptual model in Figure 3.

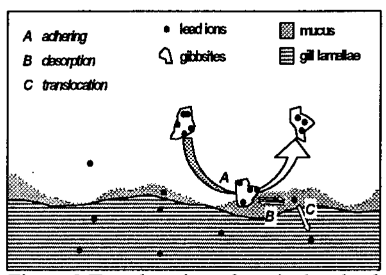

Figure 3 Translocation of particulate lead from particles into the gills

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Trace Elements in Aquatic Ecosystems and Sediments

(Technical Session 4)

TOTAL AND EXTRACTABLE LEAD IN WATER AND SEDIMENTS OF THE NAHR-IBRAHIM RIVER, LEBANON

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1. Introduction

In the Middle East Lebanon has, potentially, abundant water. Sixteen years of civil war have however left a legacy of uncontrolled pollution and haphazard and inefficient water usage. Lebanon currently depends for its domestic supply mainly on ground water that is deteriorating rapidly. Groundwater usage is badly managed while surface waters have not yet been exploited. Baseline data on the quality of Lebanese rivers are relatively non-existent. Lebanon has 15 perennial rivers. A monitoring study of most Lebanese rivers is being carried out (over three years (1996-1999) by the American University of Beirut) on the feasibility of surface water usage as drinking water. The primary objective of our study, part of the wider one, is to assess the capacity of one river for "self purification", namely the Nahr-Ibrahim river (located north of Beirut near Byblos) the catchment of which is largely underlain by limestone. The annual average precipitation for Beirut is 930 mm and 86% of this falls in winter between November and March. From March to October summer precipitation is trivial and the Nahr-Ibrahim river is fed entirely by baseflow from the limestone aquifers. The research objectives of the study comprised an investigation of the forms, distribution and likely fate of heavy metals entering the high pH water of the Nahr Ibrahim from industrial effluents. This paper focusses on the results for lead in summer and winter.

2. Materials and Methods

Five sampling sites were chosen a long a 13-km channel stretch from the confluence with the Mediterranean (site 1). The choice of these sites was based upon practicability of collecting bed load sediment and the potential for contamination from domestic waste or industrial discharges. Sampling was done in a dry season and wet season. Dates of sampling were (dry season) August 24, 1996 and (wet season,) February 26, 1997.

Bed load sediments and stream water were collected according to standard methods. Water pH, temperature and conductivity were determined *in situ* and water samples subsequently filtered through an 0.45 μm membrane in the laboratory. Sediment samples were dried at room temperature and sieved. This paper reports data for the <75 μm size. Water was analysed for major constituents and trace metals. Sediments were subjected to a sequential extraction procedure of Tessier. Total metals were extracted with hot *aqua regia*. Metals in solution were subsequently determined by flame atomic absorption spectrometry and analytical quality was assured through use of standard reference materials and appropriate replication. Water solute speciation was modelled by MINEQL+.

3. Results and Discussion

Table 1. Mean water analyses for summer and winter for samples from 5 stations: concentrations as mmol/L (Pb μmol/L)

Month	T°C	pH	Ca^{++}	Mg^{++}	HCO_3^-	Cl^-	NO_2^-	NO_3^-	NH_4^+	Pb^{++}
August	18.4	8.6	1.72	1.15	5.04	1.02	0.00012	0.015	0.019	0.190
February	12.4	8.3	1.40	0.30	4.48	0.54	0.00014	0.024	0.025	0.081

Water in a river channel comes from two general sources: mostly through baseflow from groundwater and superimposed on it periodically is storm run off. The water data (Table 1) are consistent with this scenario. The high pH and alkaline earth and carbonate concentrations are

typical of limestone aquifers. Differences between seasons can be ascribed to dilution by storm run off. In both seasons the speciation data indicated that the water was saturated with respect to dolomite and calcite and true soluble molar in February and August. In both seasons, 86.9% of dissolved Pb was present as $PbCO_3$ and 11.7% as $Pb(CO_3)_2^{2-}$ Total soluble Pb was 7.43×10^{-8} molar or 1.54 µg/L. The apparent solute excess of Pb and Ca in the <0.45µm water fraction is probably due to colloidal minerals passing the micro filter.

Table 2 records the total Pb content of the <75µm sediment fraction.Ca^{2+} was only 6.92×10^{-5}

Table 2. Pb contents (µg/g) of bed sedments (<75µm fraction) for Nahr Ibrahim sample sites.

Month\ site	1	2	3	4	5
August	112	149	110	140	76
February	54	52	44	62	35

Whereas the water chemistry data showed a general uniformity along the river and for both seasons, the sediment data (Table 2) demonstrate both site to site variations and lower values in winter (summer mean = 117 µg/g, winer mean = 49 µg/g). Summer discharge is (mean) 12.77 m^3/s and winter, 20.88 m^3/s (data from Ministry of Hydraulic and Electricity – Lebanon): In the wet season the river is reddish coloured due to its high soil-derived suspended sediment load. The river length from source (Afqua and Roueiss springs) to sea is 30 km. We interpret these data as evidence for steady sorption of Pb on to bed sediment particles during the low flow velocity conditions of the summer, when each river pool or slack behaves as a closed reaction chamber, whereas in winter sediment is transferred rapidly down river to the Mediterranean and accumulation of Pb by sorption on to an individual particle is reduced.

Table 3. Sediment Pb composition (µg Pb/g and % of total content recorded in Table 2) for 5 sites in two seasons in Nahr Ibrahim: fraction F2 is 'carbonate-bound' and fraction F4 is 'hydrous oxide-bound'.

	Fraction	1	2	3	4	5
August	F2	51 (45.5%)	70 (47.0%)	50 (45.5%)	68 (48.6%)	29 (38.2%)
	F4	27 (24.1%)	34 (22.8%)	25 (22.7%)	34 (24.3%)	20 (26.3%)
February	F2	22 (40.7%)	25 (48.1%)	20 (45.5%)	27 (43.5%)	13 (37.1%)
	F4	17 (31.5%)	17 (32.7%)	12 (27.3%)	18 (29.0)%)	13 (37.1%)

The amount of Pb in hydrous oxide-bound and carbonate-bound fractions according to the Tessier chemical fractionation at all sites is lower in February which is consistent with a fast throughput of sediment and therefore reduced sorption. However, the carbonate-dominated nature of the water chemistry is reflected in the sediment fractions since for sites 1 – 4 the carbonate-bound fraction predominates. Only in site 5 furthest upstream is there evidence for differences in sorption mechanisms. We note that the hydrous oxide-bound fraction is always important and a major role for hydrous oxides of iron in heavy metal sorption is well known (Jenne, 1968).

4. Conclusions

Speciation modelling shows that water chemistry throughout the surveyed river stretch is dominated by carbonate precipitates and this implies a safe upper limit for soluble Pb irrespective of the magnitude of incoming pollution effluents. Data from sequential fractionation of bed sediments taken together with aqueous speciation results confirm the importance of carbonate precipitates for removal of Pb from the water column and have identified a role for sorption by iron oxides. High discharge in winter may lead to all bed sediments being flushed to the sea and long term build up of Pb in bed sediments is minimised. The river water is potentially a valuable potable water resource.

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DREDGED IRON-SULFIDE RICH SEDIMENTS AT RIO DE JANEIRO LAGOON SYSTEM, BRAZIL

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1. Introduction

The lagoons of Camorim and Tijuca in Rio de Janeiro, Brazil, were dredged to improve the hydrodynamic conditions. Besides sewage sludge spills, Jacarepaguá and Camorim lagoons receive the effluents of over 200 industries, and the presence of heavy metals was formerly detected (Fernandes, 1994).The dredged sediment is a fine grained material with high content of organic matter (15-20%) and Fe-sulfides (2%). No carbonates were detected. Considering the land disposal conditions and the results of acid-base accounting tests, an analysis of the potential of acid drainage generation that takes place with the Fe-sulfide oxidation is presented.

2. Materials and Methods

The study consisted of the analysis of three samples of bottom sediments (13A, 15A, 25A), located at the discharge areas of the main tributary rivers. The total heavy metals concentration was determined by total digestion with concentrated acids. Measurements were made in flame atomic absorption spectrophotometer. The sulfur concentration was determined by LECO equipment and the organic matter was determined by chemical oxidation with potassium dichromate. Heavy metals were analysed in bulk soil samples and the percent of fines was determined by grain size analyses. Mineralogical observations were made through an electron microscope with energy dispersion system. The acid-base accounting was performed according to the method proposed by Sobeck et al. (1978).

3. Results and Discussion

Chemical analyses indicated 15-20% of organic matter and 2% of sulfur. Mineralogical analyses indicated high amounts of Fe-sulfides (Figure 1). No carbonates were detected. Table 1 summarise the major index properties for these particular samples.

Table 1 - Summary of major properties

Sample	S	SO_4	OMC	Fe	Cu	Pb	Zn	Mn	Percent of fines	
	(%)				(mg/Kg)				< 2μm	<63μm
13A	2,46	-	16,2	4,7	61	83	223	200	47	95
15A	2,09	1,02	17,0	-	41	52	111	-	73	90
25A	0,701	0,367	6,0	4,2	62	75	196	200	30	79

S – non-oxidised sulfur; SO_4 – oxidised sulfur, OMC – organic matter content in dry weight

Based on general criteria relative to land disposal of dredged sediments (IADC/CEDA, 1997), the heavy metals concentrations do not present a risk of subsoil contamination after disposal.However, short time laboratory tests (acid-base accounting) have shown that the three tested samples are located in the zone of high acid generation potential. This behaviour is due to the presence of high amounts of Fe-sulfides associated to low carbonate contents. In Figure 2 NP means the neutralisation potential and MAP means maximum acidification potential.

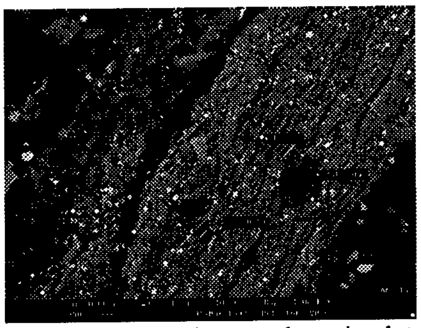

Figure 1 – Electronic microscope observation photo (sample 13A)

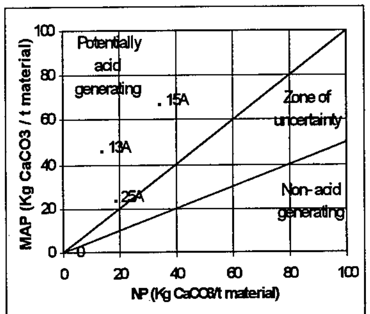

Figure 2 - Interpretation of static tests (adaptated from Sobeck et al., 1978)

According to Brannon & Myers (1994) neither hydraulic nor mechanical dredging adds sufficient oxygen to overcome the oxygen demand of polluted sediments. As a result, the dredged material in a confined disposal facility is anaerobic, except for a surface crust. The land disposal of the sediment being studied shows a peculiar feature. The sediment was temporarily placed in pond sites around the lagoons. After some time, the sediment was removed and stacked for later reutilization on local earthworks. During stacking, the whole sediment was exposed to the air and an almost homogeneous drying took place. Thus, the release of contaminants from the dredged material being studied requires the consideration of the possible effects of oxidation of the material as a whole and not only of the surface crust.

4. Conclusions

The case of land disposal of a lagoon dredged sediment in Rio de Janeiro, Brazil, is discussed. It is shown that this sediment, disposed on land and subjected to heavy O_2 exposure, can begin to release sulfates and hydrogen ions into the pore water. The hydrogen ions tend to produce a pH decrease, due to absence of carbonates. In cases of land disposal, the acidity produced in sediment itself could cause the heavy metals release, particularly those of antropic origin.

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VARIATIONS IN LEVELS AND SPECIATION OF TRACE METALS IN MINE POLLUTED RIVERS IN THE RØROS AREA, NORWAY

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1. Introduction

Residuals from mining activities are known to contribute to significant amounts of heavy metals to aquatic environments throughout the world. This leads to damaged ecosystems and food supplies, and in some instances even poisoning of humans. Many aquatic organisms, and among them fish species, are sensitive to high concentrations of metals such as Cu, Zn, Cd and Al. Norway have explored and exported huge amounts of metals, and though mining activity in Norway has now nearly ceased, residual matter still contains and contributes considerable amounts of metals and acidity to rivers, lakes, and estuaries.

Only a limited fraction of the metal species in waters are potentially bioavailable. Alkalinity, pH, content of organic and inorganic ligands and particles, and also kinetics will influence on the metal speciation, whereas especially Ca is belived to compete with metal ions for the uptake sites on cell membranes. Most readily available are probably free metal ions, and for some elements (Hg and Sn) biomethylated species. Hydroxo complexes and some other small complexes are also considered to be available to some extent, while carbonates are considered less available. Particles and high molecular weight organic bound metals cannot easily pass cell membranes and are therefore not appreciably bioavailable.

This presentation aims to give insight in the nature of the variations in metal concentrations and speciation through extreme episodes. These episodes are most important towards acute toxisity effects of metal pollution in an aquatic ecosystem. Particular focus will be placed on what time of year the higher concentrations occur and why they occur then.

2. Materials and Methods

Eight highland rivers and streams were followed through episodes of expected high metal concentrations during 1997. The dissolved metal species were fractionated by dialysis *in situ* (BENEŠ and STEINNES, 1974) through a dialysis membrane with a cutoff at 10 000-20 000 D (ca. 2-8 nm pore size). After washing and filling the dialysis bags with deionized water, they were transported to the sample sites where they were allowed to equilibrate with the river water for at least a week. Unfractionated samples were collected for total metal concentrations, and all samples were acidified to pH 1. The heavy metals Cu, Zn, Cd, Fe, and Al were determined by flame and graphite furnace AAS.

The chosen sample sites differ considerably in annual runoff and water chemistry. Discharge data in the rivers were monitored or calculated. Also monitored were pH, conductivity, alkalinity, water temperature, precipitation in the area and TOC.

3. Results and Discussion

As illustrated by Cu in the river Glåma (Figure 1), the dissolved concentrations are normally high in periods of high total concentrations. Due to slow equillibration in the dialysis bags, the

dissolved concentrations represent an integrated concentration over several days. This probably explains the somehow unregular pattern in dissolved to total concentrations.

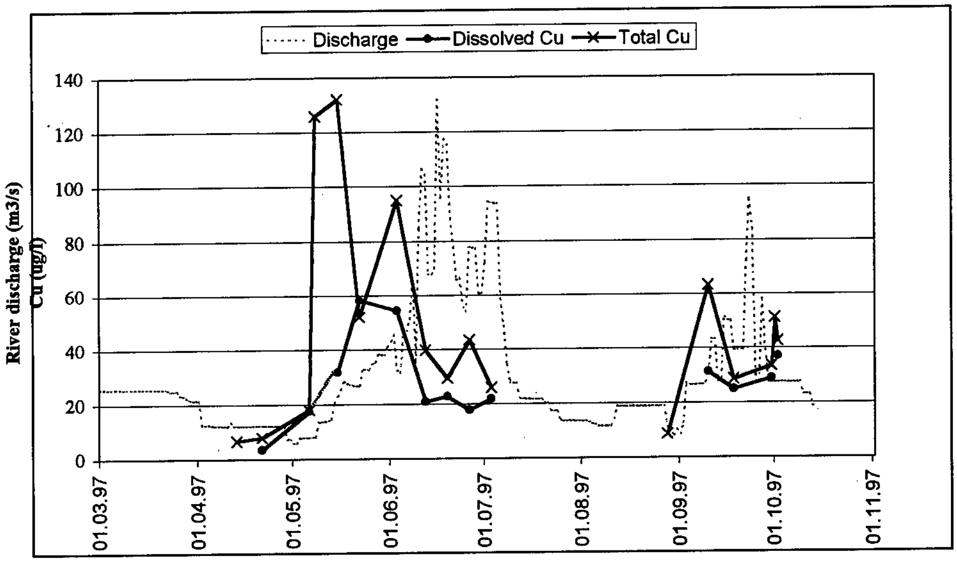

Figure 1: Total and dissolved concentrations of Cu compared to runoff in river Glåma.

In one stream the concentrations were lowest during spring flood, probably due to dilution by melt water. For most sites though, high metal concentrations were observed during spring flood, as melt water penetrated mine tailings and brought material weathered during winter to rivers and streams. The peak in Figure 1 is even higher and occurs earlier in the flood than one would normally expect. Results so far indicate that this could at least partly be caused by runoff regulation by a hydroelectric power plant, and that the metal stress during this critical period could have been decreased considerably by a different water reservoir strategy. This topic will be further discussed at the conference, along with further results on the variation of dissolved and total concentrations during the season.

4. Conclusions

Total and dissolved concentrations of heavy metals normally rise considerably during flood episodes, causing potential toxic stress and reduced populations of aquatic organisms. The extent of these episodes is probably partly regulated by human and natural reservoirs.

5. References

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SILVER CONCENTRATION IN SEDIMENTS IN THE GULF OF FINLAND: ORIGIN AND BIOAVAILABILITY

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1. Introduction

The Gulf of Finland is a part of the Baltic Sea. Silver (Ag) contamination in the Gulf of Finland arises from many different sources including the metal industry, thermal power plants, cement industry, waste incineration plants and also the photo-processing industry. Recent studies indicate that the discharge of Ag from industrial sources to air, water and land in Finland is about 0.82, 0.16 and 23 t yr^{-1}, respectively. (MUKHERJEE, 1997).

Ag concentration in the sediment of the Baltic Sea or at the coastal areas of Finland has never been measured before, although availability of Ag contributes to its toxicity to selected biota (LUOMA et al., 1995). It is also well documented that Ag^+ is highly toxic to freshwater fish (DIAMOND et al., 1990). Due to ecological problems in the Baltic Sea, considerable attention has been paid to the Gulf of Finland. In this paper we are briefly discussing the concentration of Ag in sediments collected from different depths and its bioavailability to the flora and fauna in the coastal water of the Gulf of Finland.

Sampling Sites and Load of Heavy Metals in the Baltic Sea

Our sampling sites are shown in Fig. 1. Data on airborne load, river load and direct discharge of heavy metals (except Ag) to the Baltic Sea are available since the beginning of the 1970s. Sediment chemistry in different basin areas is quite important when considering the availability of heavy metals. The simple reason is that areas of the Gulf of Bothnia are covered by primary minerals eroded from the bedrock, clay minerals, organic matter and metal sulfides whereas most parts of the Gulf of Finland are covered by primary mineral materials that are eroded from nearby shores or resuspended from shallow bottoms combined with organic materials in different quantities (H. VALLIUS, Geological Survey of Finland 1998, pers. comm.). Recently, bulk deposition of Ag in Birkenes (Norway) has been estimated to be 32 ± 11 □g m^{-2} yr^{-1} (STEINNES, 1997). Based on this value, a tentative deposition rate of Ag particulate over the Baltic Sea can be calculated as 13.2 ± 4.6 t yr^{-1}.

2. Materials and Methods

Sediment samples were taken at 11 sites during June - September 1996 using gravity cores. The cores were sliced into 2 cm thick slices to a depth of 4 cm. The deeper subsamples (also 2 cm thick) were taken at depths 14 to 16 and 43 to 45 cm. Nonmetallic spatulas were used to collect the samples, which were stored frozen in plastic bags until analysis. The samples were analyzed by an ICP-MS method. Commercial sediment reference material, Buffalo River Sediment, was used to control the analytical reliability.

3. Results and Discussion

Table 1 indicates Ag concentration in sediment samples and the calculated enrichment factors (EF) indicate that most of the values are greater than 1. In the samples we have also observed a positive correlation of Ag with Cu (r = 0.696), Pb (r = 0.557) and Hg (r = 0.818), but negative correlation with Mn (r = -0.211). The positive correlation and higher EFs suggest that Ag in the coastal sediment of the Gulf of Finland is mostly anthropogenic.

The bioavailability of Ag in sediments depends not only on its concentration but also on the presence of sesquioxides (i.e. oxides of Fe, Mn and organic materials) in the sediment layers. Each of the components may bind Ag or other metals in different ways.
Grain-size of sediments plays also an important role in the binding of metals. We have made an attempt to determine the correlation of Ag binding capacity and sediment grain size. Negative correlation ($r = -0.34$) was noted when the particle size of sediment samples is more than 74 □m, whereas particle size less than 74 □m has a positive correlation ($r = 0.26$). In addition, grain size also varies from season to season due to domination of wind velocity. This factor was also confirmed during sediment studies in San Francisco Bay (THOMSON-BECKER and LUOMA 1985). In our samples the binding capacity of Ag by fine grained sediments has been confirmed. In addition sediments contain Fe, Mn, Cu and organic matter and clay which bind Ag in different ways. It is expected that many organisms are exposed to all of these forms in their food and it has been reported that organisms select food according to the particle size (SELF and JUMARS 1978). Hence Ag in sediments of the Gulf of Finland is bioavailable to many organisms feeding on sediments and suspended matter.

4. Conclusions

In the Nordic countries, very few basic studies have been made regarding Ag in the environment. Due to increased use of Ag in the photographic industry, special attention should be made to the study of transport, fate and effects of Ag in the environment.

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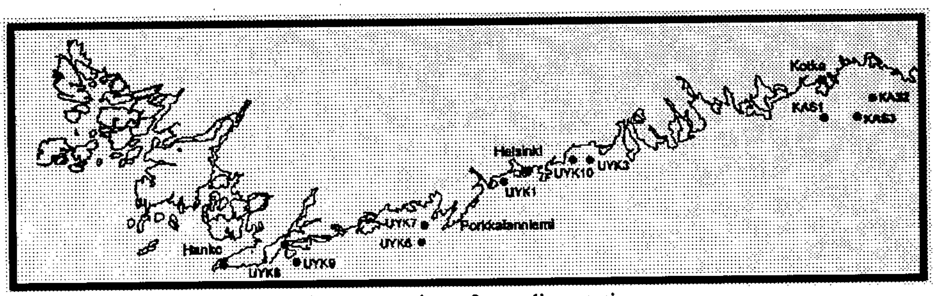

Fig. 1. Location of sampling stations.

THE MOBILISATION OF TRACE ELEMENTS BETWEEN PLANTS, WATERS AND SEDIMENTS IN AQUATIC ECOSYSTEMS

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1. Introduction

This paper reviews recent studies on the behaviour of trace elements in aquatic ecosystems.The trace elements in plants,waters and sediments determine their mobility ,flow and geochemical interactions . Balance sheets of trace elements are a useful tool to appreciate the relevance of input and output flows of ecosystem. Lakes in aquatic ecosystem are characterized as half-closed systems. Therefore,the budgets of trace elements between input and output are unbalanced and the input of many metals in aquatic ecosystem is greater than the losses .

Most lakes are made up of volcanic , sediments of lakes,therefore consist of volcanic rock or volcanic ash(lake Kusharoko in Hokkaido). The concentration of trace elements in aquatic ecosystem depends first of all on the composition of their lake sediments. Secondly,the concentration depends on a degreeof the anthropogenic influence in the environments.

The flux of trace elements into from the catchment area is greater than the weathering for all the elements. More trace elements are accumulated in lake sediments than in plants or water. However,the concentration of trace elements in aquatic ecosystems varies considerably between plants,sediments and water. The trace elements in sediments in aquatic ecosystems play a bridge function role for transfer from solid to liquid phases and to plant roots. Concentration of the metals was generally greater in plant roots than leaves or stem.

The aim of this study is primarily to show the anthropogenic influence Of the trace elements on aquatic ecoystems . Secondly, it is to show the estimated input and flow of trace elements in aquatic ecosystems.

Third,it is to evaluate the uptake of trace elements by Charophyta-flora and Macrophyten from sediments.

2. Materials and Methods

This work was conducted in several lakes in Japan among the many aspects of phytoecological studies .

Pb,Zn,Cu,Cr,Ni,Cd,As,Ca and K have been measured in sediments and plants(Charophyta-flora and Macrophyten) by atomic absorption spectroscopy, the X-ray fluorescence methods .The trace elements in the water have been determined by the X-ray fluorescence analysis with DDTC method. The uptake of trace elements by Chara braunii,chara corallina,chara globularis var.globularis,Nitella flexilisvar.flexilis,Nitella hyalina ,Scirpus tabernaemontani,Trapa bispinosa var.japonica and Phragmites australis etc. were investigated

3. Results and Discussion

The reference by Kloke et al..(1984) gave general orders of magnitude for he soil-plant transfer coefficients of a range of trace elements. The most bioavailable elements in this study were P and Mn.;the lowest relative bioavailability were Pb,As,Cr,and Cd (Alloway 1997). As Brylinsky

and Mann(1973) pointed out, it is the rate of cycling of nutrients that is important, not the concentration in the water and in the sediments.

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PHYSIOLOGICAL CHARACTERISATION OF CADMIUM UPTAKE IN WATER HYACINTH (*EICHHORNIA CRASSIPES*)

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1. Introduction

Little is known about many physiological and biochemical aspects of the processes involved in heavy metal uptake, translocation and accumulation in plants and particularly in plant species having useful characteristics for phytoremediation technology. Gaining deeper knowledge about these topics could be very useful to evaluate both the limitations of plants as heavy metal accumulators and the possible improvement of the traits required for phytoremediation (Salt et al. 1998). Water hyacinth (*Eichhornia crassipes*) is a promising plant for remediation of water from heavy metal pollution since it shows capacity to grow in water polluted with several toxic substances, to absorb different heavy metals at low concentration, a large root system, a rapid growth and a high biomass production. Here we report data about physiological characterisation of Cd^{2+} uptake in water hyacinth.

2. Materials and Methods

For the experiments using the whole plants, Cd^{2+} (as $CdCl_2$) was administered under continuous flow (600 ml h^{-1}) in 30 l tanks (9 plants $tank^{-1}$). Cd^{2+} content in the organs was determined by atomic absorption spectrophotometry (Rivetta et al. 1997a). Subapical root segments (6 mm long) were excised from roots after cutting the tips (2 mm). Cd^{2+} uptake was determined in samples (20 segments) incubated at 26 °C in 25 ml of 0.1 mM Mes-BTP (pH 6) and Cd^{2+} as indicated: then, the segments were washed twice at 2 °C in 1 mM $LaCl_3$ solution (Rivetta et al. 1997b). Transmembrane electric potential difference of cortical root cells was measured as previously described (Sacchi et al. 1997). ^{31}P NMR spectra were obtained as described by Sacchi et al. (1997).

3. Results and Discussion

Hyacinth plants of 5-15 g fresh weight were incubated for 1, 2 and 3 days in the presence of 0.05, 0.5 and 5 µM $CdCl_2$; plant growth, measured as an increase in both fresh and dry weight of roots and aerial parts, was not significantly affected by Cd^{2+}. At the highest concentration, plants absorbed up to about 30% of the administered metal (360 µmol Cd^{2+} for about 60 g of fresh weight), confirming a high capacity of *E. crassipes* to remove metal from the substrate. The evaluation of Cd^{2+} content in plant organs indicated that only about 10% of the total Cd^{2+} absorbed was translocated to the aerial part: cadmium content progressively decreased from stem to petiole to leaf blade. The higher amount of Cd^{2+} absorbed by plants was present in the roots. The low Cd^{2+} translocation might be due to the presence of molecules with high affinity for Cd^{2+} or to the compartmentalisation of the metal in the root cells. Cd^{2+} uptake in water hyacinth roots

was characterised using subapical segments. These were incubated for 1, 2 and 3 h in the presence of 0.5 or 5 μM Cd^{2+}: the uptake greatly increased up to 1 h of incubation and after this time the rate of uptake increased slowly. The transmembrane electric potential difference (E_m) of the cortical root cells was about -170 mV in the control and did not significantly change during the first hour of incubation in the presence of 5 μM Cd^{2+}. Calculation of Nernst potential using Cd^{2+} levels in the root segments and in the external medium suggested that Cd^{2+} is far from the value of electrochemical equilibrium. Subapical root segments were incubated for short times (30 min) in the presence of external Cd^{2+} concentrations in the range 1-500 μM. The kinetic of Cd^{2+} influx appeared to be saturated over 100 μM: the apparent kinetic constants $K_m = 52.2 \pm 7.5$ μM and $V_{max} = 3.0 \pm 0.3$ μmol h^{-1} g^{-1} fresh weight were similar to those of Cd^{2+} influx in subapical maize root segments (Rivetta et al. 1997b). These data indicate that the transport systems involved in Cd^{2+} influx in water hyacinth show similar characteristics to that of maize and that water hyacinth and maize accumulated similar Cd^{2+} amounts. However, Cd^{2+} appeared to be less toxic for water hyacinth than maize, as suggested from ^{31}P NMR spectra of root segments recorded in the absence or presence of 5 μM Cd^{2+}

4. Conclusions

Water hyacinth does not show particular transmembrane Cd^{2+} uptake characteristics. Its capability to remove from water high amount of the cation does not depend on a particular efficiency of the transmembrane Cd^{2+} uptake mechanism, but could be due to a higher resistance to the toxic effect of the cation.

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BIOGEOCHEMICAL ASSESSMENT OF TRACE METALS IN RIVER SEDIMENTS

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1. Introduction

Contaminated sediments have been found in almost all water bodies which have at some time received or presently receiving waste inputs from urban and industrial discharges.These contaminated sediments are now regarded as a major source of toxicants for bottom related organisms (Adams *et al* 1992). In contaminated rivers , metal exposures of biota determined by tissue residues in organisms such as benthic diatoms, can follow the down stream construction trend observed in fine grained bed sediments (Cain *et al.* 1992). Thus bioaccumulation appears to be linked to some of the same processes that control the distribution of sediment bound metals, even if the sediments are not necessarily a direct pathway of exposure. The river Kshipra (a source of drinking water supply in city Ujjain, India) is influenced by waste streams. Among the major contaminants identified are trace metals such as cadmium (Cd), chromium(Cr), zinc (Zn), lead(Pb) etc. (Bhand ,1996 Ph. D. thesis). Understanding how physico-chemical processes affect biological exposure might improve the basis for long term risk assessment from these contaminated sediments. Hence an extensive analysis of sediment samples from the tributaries of river Kshipra was undertaken to assess total and bioavailable metal concentration.

2. Materials and Methods

2.1 Sampling Strategy: The two heavily contaminated tributaries of river Khan (tributary of river Kshipra joining it after a distance of about 20 Kms.)were divided in three sediment collection zones (A_1, B_1, C_1 and A_2, B_2 and C_2 respectively for Pologround and Fort industrial area).Fine grained bed sediment and benthic diatoms (epipelic) were collected from river Kshipra at a distance of approximately 0.1, 0.5, 1.0 and 5.0 Kms. upstream and downstream of the two tributaries during Mar.-Apr. 1998 during low flow to assess site specific in metal concentration of small spatial subject. Samples of epipelic diatoms were harvested from the selected sites located approx. 10 to 20 Kms. down stream of industrial area.

2.2 Sediment procedure: Triplicate bed sediment samples were collected using Ekman dredge at each site. The sediment were immediately sieved through a 63 μm nylon mesh sieve, transported on ice box and dried in a 60°C oven. The total metals in the sediment samples were determined on filtered extracts obtained from 1g sample , digested overnight by 4 mL 70% $HClO_4$ (100°C) followed by addition of 2 ml HNO_3 till whiteresidue. The residue was dissolved in 1M HCl. Metal concentrations of analyte were determined by Atomic Absorption Spectrophotometer (Flame and Non-Flame AAS, Philips U.K.). The epipelic diatoms were harvested and analyzed by the technique described by Strőnkhorst (1994).

3. Results and Discussion

Table1 shows metal concentrations in the sediment of major tributaries of Pologround and Fort industrial area. Significant levels of Cd, Cr and Zn were found . Cd and Cr levels are attributed to metal plating industries in these areas. In terms of high Cr and Cd levels site B_1 and B_2 are found to accumulate more than other sites. Cr (24 and 60 μg/g) and Cd (14 and 11μg/g)respectively for site B_1 and B_2. Contrary to this Zn concentrations were higher in B_1 than B_2 (160 and 120 μg/g dry weight).

Table1: physico chemical characteristics of sediments i.Pologround ii. Fort industrial area

Site	pH	LOI	Cd	Cr	Zn
I A_1	8	8	9	5	110
B_1	7.6	14	14	24	160
C_1	7.8	10	10	28	128
II A_2	7.2	6	6	12	78
B_2	8.4	10.4	11	60	120
C_2	7.8	8.2	10	48	114

μg/g dry weight, LOI=Loss on ignition

Table2: concentration of metals in sediments and diatoms of river Kshipra

	LOI	%ash	Cd	Cr	Zn
I Mean	4	86	14	48	140
S.D.	0.6	2.4	6	12	24
II Mean	18.2	28	1.2	6	28
S.D.	1.6	1.8	0.08	2	8

I sediment, II diatoms S.D.=standard deviation

The results obtained for the sediment and epipelic diatom concentration of metals (Table2) indicate that Cr is potentially enriched in both sediment (48 μg/g) and diatoms (6 μg/g dry weight), than Cd (1.2 μg/g) in the river Kshipra.The diatomic concentration for metals followed the order Cd< Cr < Zn. The accumulation of Cr, Cd and Zn need to be explored further in terms of toxicity testing.

4. Conclusions

From the above studies it can be concluded that the bioaccumulation of trace metals may pose elevated exposures of these contaminant by magnification in to food chain. Also the densities of various benthic communities need to be identified in the study area for potentiality of accumulation and impact metal concentration on the population of benthos.

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CADMIUM TRANSPORT AND SPECIATION IN THE SINOS RIVER (SOUTHERN BRAZIL)

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1. Introduction

The Sinos River drains an area of 3800 km² in Southern Brazil, an area characterized by a great density of industries. The river serves as a source of drinking water for over 1.2 million habitants and, therefore water quality is of great concern. Many smelters, metalworking factories and chemical productions plants indicate that attention must be made for the trace elements (cadmium and others) distribution in the river (Baisch and Hatje, 1997). The current contribution presents the results of the study of Cd distribution along the river.

2. Materials and Methods

Water sampling was done along the river flow at water collection stations during the dry period (July 1997). The sampling was carried out by standard equipment from a boat at a central part of the river. Suspended particulate matter (SPM) was separated by water sample filtration through a filter of 0,45 μm pore size. Cadmium was determined through AAS technique in water and correspondent SPM samples.

3. Results and Discussion

The concentration of dissolved cadmium, Cd(d), steadily decreased towards the river mouth (Fig.1). Distribution of particulate cadmium, Cd(p), demonstrated the same behavior as the dissolved form, except at station 4 where river passes a zone of intensive metallurgy and the concentration of Cd(p) evidently has increased. That concentration of Cd(p) is correlated with the SPM concentration; the more SPM in water the less concentration of Cd(p). This can be explained by cadmium binding to finest particles. Station 4 was an exception to this principle where Cd(p) concentration increased with no correspondence to SPM decreasing (Fig.2).

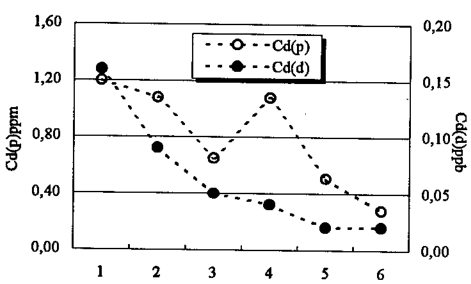

Fig.1: Distribution of Cd(p) and Cd(d) along the river.

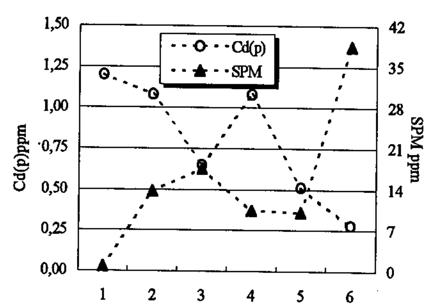

Fig.2: Distribution of the Cd(p) as function of suspension matter.

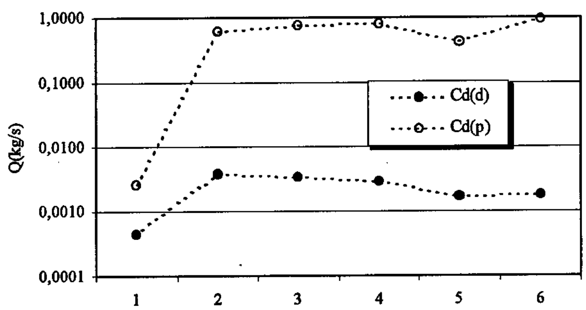

Fig.3: Influence of river discharge (Q) on particulate and dissolved metal concentrations.

The flux of Cd(d) decreased while Cd(p) increased in all with small significance towards the river mouth (Fig.3). That proves enlarging antropogenic income of Cd(p) to the river, which corresponds the results obtained by Pettine et al (1994). Nevertheless, augmentation of river discharge provides Cd(p) dissolutión and its concentration in water decreases by river mouth.

4. Conclusions

(i) Cadmium in the Sinos River is predominantly transported in particulate form, concentration of dissolved cadmium decreases towards the river mouth due to river discharge increasing.

(ii) Antropogenic cadmium contributes to the river mainly in particulate form.

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GEOGRAPHICAL VARIATIONS OF TRACE METAL ASSOCIATIONS IN SEDIMENTS FROM MAJOR RIVERS IN EASTERN CHINA

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1. Introduction

Considerable activities have been undertaken in studying the way by which trace metals are partitioned among geochemical substrates in the aquatic sediments since the 1970s, while little information is available for the geographical variations of sediment-bound trace metals. Although the aquatic sediments are characterized by their extreme complexity and diversity, their capacities for associating trace metals could be expected to have some relations to the geological backgrounds and climatic types in the river basins, since the aquatic sediments, especially the relatively unpolluted river sediments, are products of the rocks and soils within the basins during continuous weathering and leaching (Golterman et al., 1983). This paper presents the geographical variations of sediment-bound trace metals from 12 major rivers in eastern China. Both of total metals (Cu, Pb, Zn, and Cd) and their associations in various substrates in sediments were analyzed and discussed.

2. Materials and Methods

Sampling location

A total of 26 sediment samples were collected during April to July, 1992 from relatively unpolluted reaches of 12 major rivers in eastern China, including, from north to south, the Amur, Nen, Songhua, Luan, Yellow, Huai, Hanshui, Yangtze, Qiantang, Min, Bei and Pearl rivers. The basins of these rivers, which account for about 50 percent of the total area of China, cover a wide spectrum of physical geographical zones from the cold snowy forest climate and dark brown earth in the northeast to the moist subtropical climate and red earth in the southeast. At each station, the sediment was scooped directly below the water surface. All samples were contained in polyethylene bags and transported to the laboratory..

Chemical analysis

Total concentration

All samples (<63μm size fraction) were digested with a combination of HF-HNO_3-$HClO_4$ acids in Teflon beakers at 200□. The total concentrations of Cu, Pb, Zn, and Cd were analyzed by atomic absorption spectroscopy (AAS; Hitachi 180-80).

Association with various substrates

The wide-used sequential extraction procedure described by Tessier et al. (1979) was selected to study the trace metal associations with various geochemical substrates in sediments. A few modifications proposed by Chao et al. (1983), Chao (1984) and Horowitz and Elrick (1987) were taken to separate the reactive iron oxides, amorphous iron oxides and cryptocrystalline manganese oxides (Jenne, 1987), which have been considered having different intensities and capacities to bind trace metals.

The concentrations of Cu, Pb, Zn and Cd were analyzed by atomic absorption spectroscopy (AAS, Hitachi 180-80). The reactive FeO_x, amorphous FeO_x, and cryptocryystalline MnO_x were analyzed after the corresponding leaching step using AAS.

The organic matter was measured by titrimetry following dechromate oxidation. Grain size distribution was determined by a micron photo size analyzer (SKC-2000). The content of <2μm fraction was considered as the content of total clay minerals.

3. Results and Discussion

Variations of total trace metals in sediments

For all sediment samples studied, there is a large variation in the trace metal concentrations. However, all of them possess the similar geographical variations. The highest concentrations of trace metals are found in the southern river sediments. The previous work by Qu and Yan (1990) has also reported the Pearl River suspended matter contains higher concentration of trace metals than the Yangtze and Yellow river sediment. As noted by them, we suggest this southern enriched trace metal pattern in suspended matter and sediments is the result of higher trace metal geological background (non-ferrous deposits are widespread in this region).

It should be noted that the contents of trace metals in the Yellow River sediments are lower than both of those in the southern and northern rivers. Unlike any other rivers of china, 90% of the total sediment load of the Yellow river originate from the loess in the middle reaches of the river (Zhang et al., 1990a) which contains a very low level of organic matter, clay minerals, as well as iron and manganese oxides. Because of its highest sediment load in the world. We believe this low level of trace metals in the yellow River sediment due to the loess could not be neglected when the global average composition of trace metals in river sediments is discussed, as we noted earlier (Chen and Wang, 1996).

Variations of trace metals associated with the primary mineral phase

Considering the sequential extraction procedure in this paper used to define the trace metal associations, the trace metals found in the residue are notably fixed within the crystalline lattice and herein are considered to be fragments of the primary mineral phase. Accordingly, all the other fractions involved materials formed through physical and chemical processes of weathering of the primary minerals are of the secondary mineral phases.

The contents of trace metals in the primary phase mainly depended on the weathering degree of the primary minerals in sediments. The highest percentage contents of trace metals in the primary phase are found in the Yellow River, followed by the northern and then the southern rivers, exactly the contrary of the geo-chemical variations of total trace metals. This regional variation is coincident with the weathering strength of the primary minerals in eastern China. Except for the Yellow River Basin, both precipitation and temperature increase from north to south in the country. As a result, the intensity of chemical weathering of the primary minerals increase from north to south, and therefore the trace metals remained in the residue are less in the southern river sediments. Although the temperature in the yellow River Basin is higher than in the northern rivers, the precipitation is much less. Inn fact, the Yellow River Basin is one of the driest regions in China. The little precipitation limits the chemical weathering in the Yellow River sediment, and therefore a large proportion of trace metals remains in the primary phase.

Variations of trace metals associated with the secondary mineral phases

Different trace metals show the different variations with the secondary mineral phases, Cu is mainly associated with organic matter and the amorphous iron oxides, while Zn with the amorphous iron oxide phases and carbonate, Pb with iron and manganese oxides, and Cd with the cryptocrystallie manganese oxide, reactive iron oxides and carbonate. It should be noted that Cu, Zn and Pb all have a large proportion partitioning in the amorphous iron oxides which reveals that amorphous iron oxides is an important sorption sink for trace metals. Organic matter is also very important to Cu and Cd.

The large proportion of exchangeable and carbonate-bound Cd and Pb is found in the Yellow River sediments. Again, it may be related to the carbonate-rich loess in which carbonate concentration ranges from 5% to 20% (Zhang et al., 1990b). Wang et al. (1994) has also reported that the trace metals associated with carbonate are much higher in the soils of the Yellow River delta than those of the Yangtze River and Pearl River deltas.

The soils of the northern river basins in China, e.g., the Amur, Songhua and Nen rivers basins are richer in organic matter. The highest content of organic matter is also found in corresponding sediments. As a result, from the Yellow river to the Amur River sediments, the contents of trace metals associated with organic matter increase. Some of the southern rivers, e.g., the Min River, which flows from the well-vegetated Mountain area, also contain a high proportion of trace metals associated with organic matter.

The geographical variation patterns of trace metals associated with reactive and amorphous iron oxides and manganese oxides are not very clear from north to south. It is probably because the contents of iron and manganese oxides in sediments are controlled by the redox potential (Eh) which is affected by many complicated factors such as the groundwater level, as well as the weathering process.

ACCUMULATION OF HEAVY METALS IN SEDIMENTS: TEMPORAL VARIATION.

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1. -Introduction

Sediments form biologically important habitats for a wide variety of freshwater, estuarine and marine organisms, many of which provide a source of food for man. Through natural and anthropogenic events, metals often accumulate to high levels in sediments, and may be incorporated in the biota. Not only can this be detrimental to organisms, but it may also increase the flux of metals into aquatic food chains.

An understanding of the nature of sediments and the geochemical behaviour of metals is critical to an understanding of the biological availability of metals in sedimentary environments.

Organic and inorganic material settle out of the water column onto the sediments, bringing with them contained metals. Flocculation and subsequent precipitation may also strip out dissolved and colloidal metals. In the sediments, metals are partitioned among various sinks; this partitioning will depend on such physicochemical variables as the nature and concentration of ligands in the ambient water, the nature and concentration of the solid substrates, the pH and the redox potential Campbell and Lewis (1988).

The aim of the present work is provide information on the levels of Cu, Mn and Zn contents in the surface sediments from two rías (Galicia, España) and the variation along twelve months.

2. -Materials and Methods

96 sediment samples (0 to 30 cm) were collected at nine sample stations , five stations in a polluted area, Ferrol Ría and four stations in an unpolluted area, Cedeira Ría (Galicia, Spain). The samples were collected using a box corer. The collection of samples was carried out each month, during 1994/95 in each station.

The carbon content was estimated by the Sauerlandt method, modified according to Guitián and Carballas. Available sediment metals were extracted with NH_4Ac (pH 7). The method followed for total analysis of metals consisted: dissolution with oxiding acid such as HNO_3: HCl (1:3 v/v) in combination with FH, adding boric acid to eliminate the excess HF Loring (1987). The digestion was carried out in microwave oven; the extracts were analysed by atomic absorption spectrophotometry. A statistical treatment of the results was carried out using the program SPSS/PC+, analysing first of all the correlations which exist and their statistical significances and secondly one and two way variance analysis (ANOVAS).

The efficiency of the procedure of extraction and analysis was controlled analysing international standards from estuaries such as MESS-1 which come from the Marine Analytical Chemistry Standards Program of the Canadian National Research Council (Ottawa, Canada).

3. -Results and Discussion

The content of Cu, Pb and Zn in both Rías appears different as showed in table 1. Higher metal content was determinated in Ferrol Ría than Cedeira Ría; this may be attributed to the main

sources of pollution in Ferrol Ría are wastes from a smelter industry and to the higher organic matter content.

Table 1. - Available Cu, Pb and Zn content (mg. kg^{-1}) in Ferrol and Cedeira Rías.

Sample	Metal	Nov.	Dec.	Jan.	Febr.	Mar.	April	May	June	July	Aug.	Sept.	Oct.
FS1	Cu	8	5	9	7	12	7	6	10	8	7	9	13
	Zn	133	224	101	188	206	214	191	203	143	191	187	188
	Pb	40	69	68	31	54	89	84	31	52	50	52	87
FS2	Cu	7	1	16	5	5	4	5	1	12	6	2	4
	Zn	45	68	96	45	60	60	74	21	44	24	51	40
	Pb	7	2	8	7	3	2	5	7	6	4	4	5
FS3	Cu	5	7	12	4	5	8	8	6	11	9	10	4
	Zn	53	100	95	11	100	34	74	32	11	84	57	15
	Pb	2	1	8	2	2	4	3	4	3	3	2	1
FS4	Cu	11	12	13	17	6	7	6	16	12	12	16,5	13
	Zn	229	247	243	223	217	215	222	240	206	219	198	235
	Pb	106	118	97	100	117	38	96	101	112	89	96	102
CS1	Cu	6	5	8	3	5	6	4	8	4	4	5	4
	Zn	6	10	2	6	3	6	10	2	6	8	6	2
	Pb	5	5	3	4	2	3	4	2	6	7	4	5
CS2	Cu	5	9	10	6	5	11	14	9	4	4	6	5
	Zn	9	4	2	19	8	29	7	4	11	4	11	11
	Pb	2	1	2	2	3	4	4	3	3	4	3	4
CS3	Cu	5	8	9	2	3	3	7	8	2	4	8	3
	Zn	3	1	4	2	1	2	2	1	2	3	2	2
	Pb	5	2	7	3	2	1	2	1	3	2	3	1
CS4	Cu	9	8	2	3	2	4	2	7	8	12	9	9
	Zn	3	2	2	1	2	2	2	1	4	4	3	3
	Pb	4	7	3	3	3	2	4	3	2	3	3	3

4.- Conclusions

Metal concentrations range in low levels for all samples, however the highests Zn, Pb and Cu levels are found in the Ferrol Ría sediments where anthropogenic inputs are more importants.
The smelter industry's effluent discharge can be implicated in increaseing the levels of Pb and Zn en SF4 sample(moderate contamination).
Irregular temporal variation occurs in both Ferrol and Xubia areas.
The total and available content of metals indicates that Ferrol Ria is a moderate polluted stuary whereas Cedeira Ria is unpolluted area.

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HG FORMS IN NATURAL, URBAN SOILS AND BOTTOM SEDIMENTS OF THE MIDDLE AMUR

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1. Introduction.

The importance of organic matter in speciation and fate of Hg in the environment is widely known. The purpose of the work was to examine the content of Hg bound to basic fractions of organic soils and river bottom sediments, such as fractions of humus and inorganic matter.

2. Materials and Methods

The Hg content in different fractions of natural and urban soils, and the Middle Amur bottom sediments was investigated. Mountain lessivied brown forest soils of lowlands' setting and forest podbels (brown-podzolic) of plane clayey river terraces are widely distributed in the area. Their urban analogues subjected to anthropogenic impact were investigated in the City of Khabarovsk. Bottom sediments were sampled in 4 sites of the middle and lower stream of the Amur River. The range "brown forest soils → forest podbels → bottom sediments" should be considered as a landscape system united with substances' water migration ("conjugate landscape" after Polynov, 1934).

The fractionation outline applied was as follow: extraction of humin acids with $Na_4P_2O_7$ followed separation onto humic acids (HA), fulvic acids (FA) and free fulvic acids (fraction 1a) (FFA) were carried out with standard methods of soil chemistry (Kononova and Belchikova, 1961; Orlov, Grishina, 1981); extraction of "not-organic" Hg fraction (Zyrin et al., 1981); $(NH_4)_2$-EDTA pH3.0; H_2O. The organic extracts were wet-digested with H_2SO_4 and $KMnO_4$. Analysis was made with the standard balanced "cold vapour" AAS. The total Hg content was also determined (Dumarey et al., 1987). Content of the total organic Hg and Hg bound to humin ("not-hydrolysing residue") was counted from corresponding ratios.

The analysis sensitivity was 0.002 ppm.

3. Results and Discussion

Most of the soil and bottom sediments Hg turned to be bound to humic acids and humin (Table 1). These fractions are the principal Hg deposit pool in the investigated soils and bottom sediments and, in general, in the landscape migrating system. A smaller Hg portion was found in mobile fractions of humus - fulvic acids and free fulvic acids. The portion of Hg-FA and Hg-FFA is comparative to Hg-humin and Hg-HA for the urban soils. The urban soils turned out to be polluted with Hg to much more dangerous level than it could be concluded from the total Hg determination.

The content of Hg bound both to mobile fractions and HA increases considerably and, on the contrary, the content of Hg-humin decreases within the system. $Na_4P_2O_7$ solution extracted nearly all of the Hg bound to humus of the bottom sediments. This indicated that two contradictory processes took place in the bottom sediments. Such processes are of great importance in regard to fate of Hg compounds in water ecosystems.

Table 1: Mean content of Hg in fractions of soil *A* horizon and river bottom sediments upper 0-5 cm layer, ppm

Fraction (extract)	Brown forest		Forest podbels		Bottom sediments
	natural	urban	natural	urban	
H_2O	0.00	0.00	0.00	0.00	0.01
$(NH_4)_2$-EDTA	0.00	0.00	0.00	0.00	0.01
Free FA (fraction 1a)	0.00	0.02	0.00	0.01	0.03
Fulvic acids	0.01	0.02	0.01	0.02	0.06
Humic acids	0.06	0.10	0.05	0.06	0.13
Humin	0.16	0.16	0.12	0.10	0.02
Total organic matter	0.23	0.30	0.18	0.19	0.24
"Not-organic"	0.00	0.01	0.01	0.03	0.01
Total	0.23	0.31	0.19	0.22	0.25

The ratio Hg-HA/Hg-FA turned out to be stable within the entire profile of both natural and urban soils (5:1) and the bottom sediments upper layer (2:1). This proves the existence of a correlating and dynamic system of Hg organic compounds in soil and bottom sediments. Probably, the content of Hg fractions may be used for indication of soil and bottom sediments' matter transformation and migration.

4. Conclusions

In general, fate of Hg in the investigated soils and bottom sediments is connected with organic matter: humic acids and humin are the principal Hg deposit milieu, whereas fulvic compounds are the main mobile ones in landscapes. Two contradictory processes take place in the bottom sediments resulting in accumulation of both mobile and immobile Hg fractions at the expense of Hg-humin one.

The Hg content bound to mobile compounds increases considerably in urban soils, which seem to be polluted to much higher level than it could be concluded from the total Hg content analysis.

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TRACE METAL DISTRIBUTION IN SEDIMENTS OF NORTHERN DWINA ESTUARY, RUSSIA

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1. Introduction

The Northern Dwina river flowing into the White sea is one of the major rivers of the North-Western Arctic. Its annual river discharge is 109 km^3/year and total annual drifts discharge is 4,5 mln.tons. Its estuary river is a very important economic zone, where timber, pulp and paper and shipbuilding industries and water transport concentrate, which causes a high level of anthropogenic pollution (Artemyev, 1996). The study of processes related to chemical behavior, transport and accumulation of pollutants is particularly important for managing the impacts of human activities and sustainable management of such critical areas. This study presents information on heavy metal distribution in surface sediments along a transect through Northern Dwina estuary.

2. Materials and Methods

Sampling of surface sediments was carried out by grab core "Ocean" at 11 stations along the salinity gradient in Northern Dwina Bay of the White sea on board MS "Prof. Vladimir Kuznetsov" during the scientific cruise in August 1996 within the framework of INTAS project 94-391 "Biodiversity and Adaptation Strategies of Arctic Coastal Marine Benthos". In the laboratory the fine-grained sediment fraction (<63 μm), separated by seaving from upper geomorphological sediment layer samples was subjected to total acidic dissolution with determination of Zn, Co, Cu, Ni, Pb, Mn, Fe and Al contents by ICP AES method (Totland et al, 1992).

3. Results and Discussion

Results on surface salinity (S_{surf}) and metal contents in surface sediments are given in table 1.
In order to study interelemental associations, the correlation coefficients of the elements analyzed were computed. Co, Cu and Ni exhibit strong correlation to macroelements Fe and Al ($r>0,8$). Zn correlated significantly with Pb ($r=0,78$). Mn displayed weak correlation with other elements studied ($r<0,3$).
Basing on correlation analysis data and on expressing trace metals by their element/Al ratio the conclusion was made, that Co, Cu and Ni in estuarine sediments are associated with clay minerals and Fe-oxyhydroxides. The main source of these elements appear to be natural terrigenous. For Zn and Pb the presence of anthropogenic input was indicated. Special distribution of Mn was related to sensitivity of this element to local oxidizing condition in surface sediments at sampling stations.
In the Northern Dwina estuarine mixing zone going from river to the open sea by salinity gradient (from station 1 to 11) metal enrichment is observed at stations 1 and 8-9, which probably mark, respectively, river-sea and sea-river boundaries with "frontier" salinities of 0 and 20 ppt, where intensive processes of flocculation and sedimentation lead to metal accumulation in sediments. This is due to high horizontal stratification of water mass in Northern Dwina

estuary (Artemyev, 1996), where a part of river material is precipitated within the very low salinity region of estuary, while most river sediment is carried to the sea with the river water in the upper layer underlayed by a salt wedge (Dyer, 1991; Morris, 1986), being precipitated when the zone of vertical water mixing is reached.

Table 1: Metal and organic carbon contents in surface sediments of Northern Dwina estuary

№ st	S_{surf} (ppt)	Al (%)	Fe (%)	Mn (ppm)	Zn (ppm)	Pb (ppm)	Co (ppm)	Cu (ppm)	Ni (ppm)
1	0	5,7	3,5	674	159	28,1	20,4	21,7	49,0
2	0,5	5,6	2,9	610	61,7	6,8	18	13,1	33,7
3	7	5,7	3,0	718	80,3	18,4	19,6	15,9	32,5
4	9	5,3	2,9	602	71,5	14,2	18,8	13,5	27,7
5	7	5,0	2,5	633	67,7	16,2	16,2	12,2	24,5
6	13	4,9	2,5	603	61,0	13,3	15,6	10,5	23,8
7	18	4,9	2,2	403	80,5	4,7	14,5	9,6	19,9
8	20	4,7	1,9	364	161	47,6	12,9	9,5	18,1
9	20	5,9	3,2	474	42,8	15,4	19,9	16,5	34,1
10	21,5	5,2	2,4	389	54,2	16,3	15,6	11,8	24,9
11	22	4,7	2,6	663	69,9	26,0	15,2	11,5	13,1

4. Conclusions

Water mass stratification plays a key role in distribution of metals in estuarine sediments. Within the Northern Dwina estuarine geochemical barrier two subbarriers can be indicated, where intensive biogeochemical interactions take place.

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DISTRIBUTION OF HEAVY METALS IN PARTICLE SIZE FRACTIONS OF SEDIMENTS OF THE KEELUNG RIVER (TAIWAN)

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1. Introduction

Due to high industrialization and discharge of domestic wastewater, there is an increase in the amount of heavy metals in the rivers. Most of the discharged metals become part of the suspended matter, and hence they contribute to the metal contents of the sediments. As a result of their accumulative, toxic, and stable nature, heavy metals in sediments may serve as indicators of pollution of a territory (1). Heavy metals in sediments are significantly affected by physico-chemical and granular compositions. The non-uniform distribution of trace metal over the range of grain size fraction causes variations in the metals contents of sediment samples, even within the same area. The objective of this study was to determine the distribution of various heavy metals in the bottom sediments of the Keelung River and to access the effect of grain size on this distribution.

2. Materials and Methods

Bottom sediments were sampled from nine sites along a section (0.9 to 90 km from riverhead) of the Keelung River in August 1997 and March 1998. Sediment samples were separated into two size fractions, coarse (<0.84 mm) and fine (<63 μm). Then these two fractions were digested with HNO_3-HCl-HF for determination of Pb, Cu, Zn, Cr and Al by flame atomic absorption spectrometry (AAS) (2). Metal concentrations were normalized to the concentration of 'conservative' elements, i.e. aluminum to eliminate the effect of grain size on the concentration of metals in sediments.

3. Results and Discussion

It has been found that heavy metals were enriched in both the sediment fractions and they tended to accumulate in the fine fraction of sediments because of their greater surface area. However, the concentrations of heavy metals in the coarse and fine sediment fractions (Figure 1), had similar distribution trends (Figure 2). These results revealed that the geochemical fate of heavy metals in the Keelung River were the same. . Concentrations of heavy metals in both size sediment fractions increased with increasing distances from the riverhead. On the other hand, comparison with 'conservation' elements, the ratios of metal/Al, could reflect the extent of metal pollution in the sediment for each reach of the Keelung River more clearly without effects of grain size on metal contents.

4. Conclusions

Because of the particle sizes, heavy metals of sediments distributed similarly in the Keelung River, decreasing with increasing flowing distance. It is possible to assess the degrees of pollution of sediments in Keelung river by determining the metal contents in coarse or fine sediment fractions.

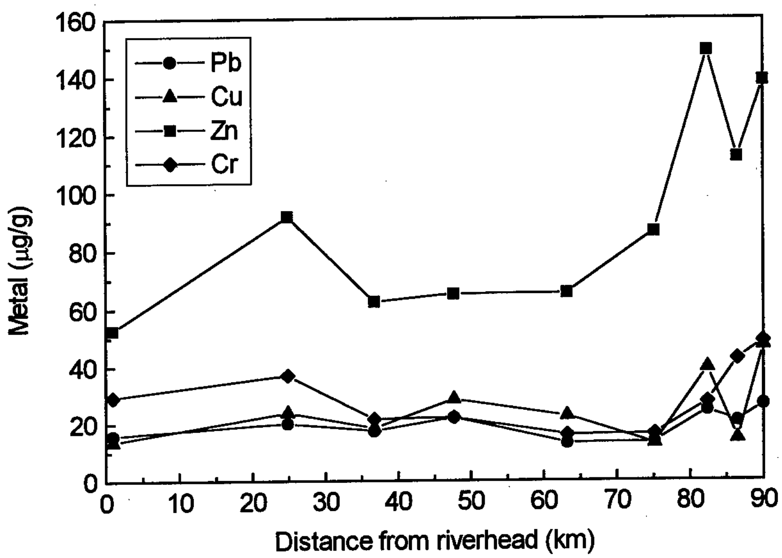

Figure 1: Longitudinal profiles of heavy metals in coarse fraction of sediments of Keelung River.

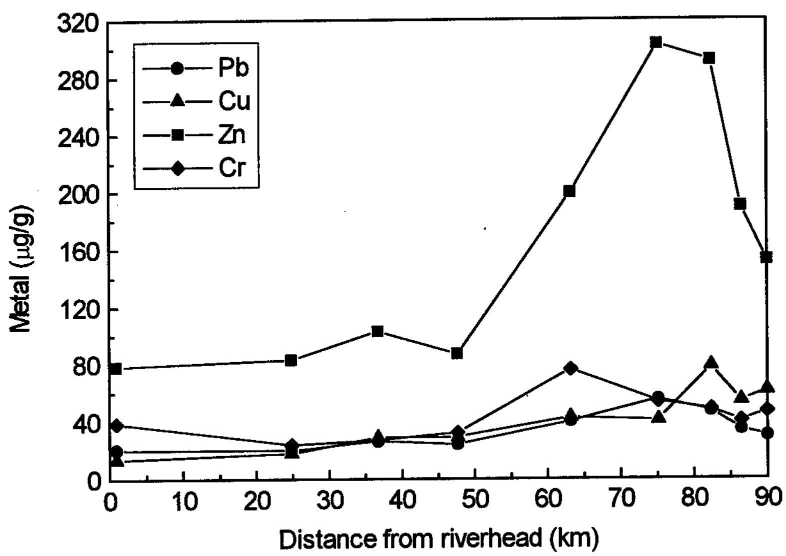

Figure 2: Longitudinal profiles of heavy metals in fine fraction of sediments of Keelung River.

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ARSENIC DISTRIBUTION IN SURFACE SEDIMENTS OF SACO DO ENGENHO INLET AND DISPERSION TO SEPETIBA BAY, RJ, BRAZIL

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1. Introduction

On a global basis, the principal direct sources of As into aquatic systems include domestic and industrial wastewater, electric power plants, base metal mining and smelting, and atmospheric fallout of contaminated aerosols (Nriagu and Azcue, 1990). Indirect sources include leaching of slag heaps, ore bodies, and the residues of pesticides and fungicides from soil.
Heavy metal contamination in Sepetiba Bay had already been widely studied by several authors that considered it as moderately contaminated by these elements (Lacerda, 1983; Pedlowsky, 1990; Barcellos, 1991; Magalhães, 1991; Magalhães *et al*, 1993; Magalhães *et al*, 1994; Karez *et al*, 1994). Magalhães & Pffeifer (1995) observed very high As concentrations in sediments near a metallurgical plant in an inlet (Saco do Engenho) inside Sepetiba Bay. The main objective of this work is to study the As distribution and dispersion along the Sepetiba Bay sediments.

2. Materials and Methods

The samples were collected with the help of a plastic shovel in shallow stations and a Vanven grab in deep stations. All samples were packed in plastic bags and kept in ice during the transport to the lab. In the laboratory the samples were sieved with a 63μm pore sieve and dried at temperature no higher than 60^0C, steeped and kept for further analysis.
This work determined the weakly and strongly bound As fraction. The weakly bound, fraction was determined through the extraction of 0.25g sediment with 10 ml of 1 M HCl for 24 hours. The strongly bound residual fraction was determined using 0.1g of sediments with 10 ml of concentrated HNO_3 in a close recipient at 130 °C for 15 hours. All samples were filtered and pre-reduced with KI. The solutions have a final KI concentration of 10%. After three hours of pre-reduction, As was analyzed using an atomic absorption spectrophotometer VARIAN-1475, with Hydride Generation VGA-76, under conditions specified by Magalhães and Pfeiffer (1995).

3. Results and Discussion

The mineral used in the factory contains, in its strongly bound fraction, 63 $\mu g.g^{-1}$, and 13 $\mu g.g^{-1}$ in its weakly bound fraction. Factory's main waste (400 $ton.d^{-1}$) disposed before using As_2O_3 in the production of Zinc lingots, presents 55 and 39 $\mu g.g^{-1}$ in strongly and weakly bound fractions respectively. After using As, a new waste is formed, this secondary waste showed very high As concentrations in the both studied fractions. Strongly bound fraction presented values up to 63,100μg $As.g^{-1}$ and the weakly bound fraction 52,700$\mu g.g^{-1}$.
As in sediment samples presented a general trend tom decrease its' concentrations towards the Sepetiba Bay. The Saco do Engenho Inlet sediments, where the Industry Waste Output is located, presented the highest values (up to 607 $\mu g.g^{-1}$in strongly bound fraction). Sediments inside Sepetiba Bay presented concentrations around the world average concentration (10 $\mu g.g^{-1}$)

for the weakly bound fraction, while in strongly bound fraction As content reaches up to 50.4 $\mu g.g^{-1}$.

The results clearly showed that the As, found at Saco do Engenho Inlet and Sepetiba Bay, comes from the Industry. Even the mineral used by the industry (before utilization of As_2O_3 to produce Zinc lingots), presented reasonable concentration of As. On the other hand, the secondary waste released after the use of As_2O_3, is highly contaminated with this metalloid. The reject pile is located in open air, bordering a brook that flows into Saco do Engenho where the samples were collected. It means that rains, winds and lixiviation processes is probably spreading As high concentrated waste all over the area, reaching Saco do Engenho Inlet sediment in very high concentrations.

4. Conclusions

A general trend was observed in the As distribution and dispersion, the sediment concentrations decrease from the Saco do Engenho Inlet, where the industry waste output is located, to the Sepetiba Bay. This trend strongly suggests the Industry as the main source of As to the Sepetiba Bay area. All sediment samples presented high As concentrations in both studied fractions. Sepetiba Bay samples showed As contents three to five times higher than the world average concentration in the strongly bound fraction, although the weakly bound fraction values were under world average concentration. The results of weakly bound fraction strongly suggests that this metal is probably entering in the sea food web, although other studies must be performed to identify this speculation.

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COPPER PARTITION FROM SEDIMENTS TO WATER: RESULTS OF SUSPENSION EXPERIMENTS

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1. Introduction

The Patos lagoon in its estuarine part is strongly affected by the influents containing the trace elements. As an example, the last accidental leakage of about 9,000 tones of the technical sulfuric acid in September 1998 led to the copper contamination of estuarine sediments. These contaminated sediments represent an important factor of water pollution as they periodically suspended by waves and currents, and, also, due to the dragging of the navigation channel.

In this paper, we present the results of suspension experiments aimed at evaluation of channel dragging effects on copper partition from the polluted sediments to water.

2. Materials and Methods

The laboratory suspension experiments were conducted with sediment samples (control and duplicate) taken from pristine area of the estuary. The control sediment sample contained 25 mg/kg of copper, while its duplicate was spiked by copper hydroxide up to the total copper concentration of 200 mg/kg. In the experiments, we used deionized water. The ratio (water depth)/(sediment layer thickness) was 8:1 and corresponded to the actual ratio in the dragged channel, i.e., 12.0 m/1.5 m. Copper concentrations in the liquid phase were measured at time intervals from 3 hours to 8 days during the 20 days-experiments. The ultra-centrifugation was used for liquid phase separation, and AAS technique was used for determining copper in the samples.

3. Results and Discussion

In the control experiment, pH of the solution was approximately constant during the experiment, at the level of 8.8. The copper concentration in the solution varied from 0.37 μM/l to 0.55 μM/l, with the average of 0.40 μM/l. The latter is fairly close to the equilibrium dissolved copper concentration (i.e., 0,63 μM/l which corresponds to the equilibrium with solid copper hydroxide at this value of pH). The concentration of dissolved organic carbon (DOC) also did not demonstrate considerable changes, varying in the range of 53-56 mg/l. The conductivity of the solution increased slowly during the experiment, from 1000 μs after 3 hours of suspension to 1075 μs at the end of the experiment. On the whole, the control experiment demonstrated quite high stability of the sediment–water system, as well as the relative equilibrium of the dissolved components.

The experiment with suspension of spiked sediment showed quite different composition of liquid phase. Only pH values were the same as in the control experiment. After the first 24 hours of suspension, the copper concentration in the solution reached its maximum (22,2 μM/l), which was about 60 times higher than that in the control experiment. Then, the solution copper concentration decreased, and reached the value of 5,31 μM/l at the end of the 20 days experiment (Fig. 1). The changes of the DOC concentration resemble those observed for copper. The DOC concentration also reached its maximum (66 mg/l) at 24 hours and then decreased to 43 mg/l.

Contrary to the copper and DOC behavior, the conductivity demonstrated steady increase during the experiment.

The obtained results demonstrate strong partitioning of copper from sediments to water, even in low-alkaline conditions. The similarity in behavior of DOC and copper concentrations during the experiments suggests that the liquid phase copper in our experiments was presented in the form of copper-organic complexes. The decrease in the dissolved copper concentration during the experiment with spiked sediment sample was, presumably, due to aggregation of organic molecules and complexes of copper-organic substance to bigger particles, and their subsequent deposition.

4. Conclusions

The main conclusions from our study are:

(i) suspension of copper-contaminated sediments of the Patos lagoon, when dragging, can lead to strong water contamination with copper;

(ii) copper can preserve its high concentration in water during long periods, that may affect aquatic organisms.

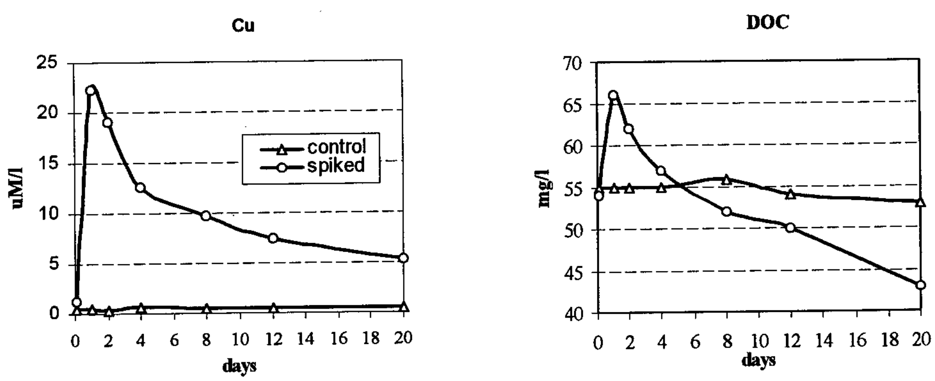

Fig.1 Changes of the solution copper and dissolved organic carbon within the suspension experiment for control and spiked by copper sediment samples.

PRELIMINARY ASSESSMENT OF WATER FERN (*SALVINIA*) PLANTS FOR UPTAKE OF CD, AL, MN, AND FE

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1. Introduction

Cadmium (Cd), aluminum (Al), manganese (Mn), and iron (Fe) have been identified as potentially toxic metals in aquatic food webs and are known to occur in coal pile runoff (Adriano, 1986). In general, a characteristic of vascular plants is that they accumulate metals and other elements in excess of their physiological need (Epstein, 1972). This characteristic has proven useful in constructed wetlands designed to take up pollutants and thereby filter pollutants from waste waters (Kadlec and Knight, 1996). Surface waters receiving runoff from coal piles or fly ash piles at the U.S. Department of Energy's Savannah River Site often have elevated concentrations of trace elements. The main objective of this study was to investigate the potential of *Salvinia rotundifolia* for its tolerance to elevated concentrations of Cd, Al, Mn, and Fe similar in coal pile runoff. From this we anticipate that we should be able to create predictive models for plant tissue concentrations in plants exposed to metals in constructed wetlands.

2. Materials and Methods

Salvinia plants were grown for 7 days periods in modified 0.10 *M* Hoagland nutrient solution(Hoagland and Arnon) as a pretreatment. After a 7 day pretreatment period, selected plants were transferred into 2L plastic pots containing 1 L of fresh 0.10 *M* Hoagland nutrient solution and various treatment concentrations of Cd, Al, Mn, or Fe in different experiments. The 0.10 *M* Hoagland nutrient solution contained nutrients in concentrations (mgL^{-1}), of approximately: 21.0 N, 3.1 P, 23.4 K, 20.0 Ca, 4.9 Mg, 6.4 S , 0.025 6, 9.028 Mn, 0.001 Cu, 0.0005 Mo, and 0.0024 Zn, 2.0 Fe, and 67.3 C. The Fe source was Fe-330 Sequestrene (Ciba-Geigy Companby, Ardsley, NY). Carbon was added as $NaHCO_3$. The experiment was conducted in a green house with 15 hrs day and night mean temperatures 31± 3^{0}C and 23±2^{0}C respectively. The metals were applied as $CdSO_4$, $AlSO_4$, $MnSO_4$ or $FeSO_4$. The treatments were selected to be within the plant tolerance limits of each metal based on preliminary acute toxicity experiments. For Cd, the treatment concentrations were 0.0, 0.1, 1.0, 5.0, or 8.0 μg Cd L^{-1}, Al treatment concentrations were 0, 0.191.0, 10.0 or 100.0 μg Al mL^{-1}, Mn treatment concentrations were 0,1.01 5, 10, or 100 μg Mn mL^{-1}. Iron treatment concentrations were 0,1, 10, 50, or 100 μg Fe mL^{-1} Solutions were replenished daily with de-ionized water to avoid depletion by evaporation. The concentrations were similar to those occurring in wastewaters in the Savannah River Site coal pile basin. The experiment was conducted in a randomized complete block design with three replications. Plants from each pot were harvested after 7 days and after 14 days. The experiments were repeated three times. Harvested plant material from each trial was carefully rinsed, oven dried to constant weight, weighed, ground and analyzed for various metals and other nutrients by Inductively Coupled Plasma Optical Emission Spectrometry.

3. **Results and Discussion**

Treatment concentrations of Cd greater than 8 µg Cd mL^{-1} were toxic to *Salvinia*. However, toxic limits of Al, Mn, and Fe treatments were above 100 µg mL^{-1} These preliminary findings suggest that *Salvinia* could be a likely candidate to use in treatment wetlands for amelioration of effects from coal pile runoff. Furthermore, *Salvinia* would play an important role in short-term movement of these elements in aquatic food webs. Long-term studies are needed to quantify the elemental concentrations in coal pile runoff waters below which significant uptake in aquatic, vascular plants would not occur. In this presentation the potential for Cd, Al, Mn, and Fe uptake by other aquatic plants such as *Juncus, Wolffia, Lemna, Azolla, Myriophyllum, Ceratophyllum,* and *Eleocharis* will also be discussed.

Table 1: Elemental concentrations in *Salvinia* tissue.

Treatment conc. (µg mL^{-1})	Cd	Al	Mn	Fe	Dry wt. (Fe Expt.)
	------(µg g^{-1} tissue dry wt.)------				
0	79	134	75	1140	1.67
0.1	10	589	NT	NT	NT
1	21	2199	333	1325	1.72
5	1638	NT	1819	NT	NT
8	3287	NT	NT	NT	NT
10	toxic	3989	2437	6797	1.25
100	toxic	20410	17560	43000	0.66

NT=No Treatment

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EXTENT OF PYRITIZATION OF TRACE METALS IN SEDIMENTS FROM GUAYMAS BASIN, GULF OF CALIFORNIA

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1. Introduction

Under reducing conditions, Fe and Mn oxyhydroxides are reduced and solubilized to Fe(II) and Mn(II) species, respectively. A portion of these two dissolved constituents in sediments can diffuse upward until they are oxidized and reprecipitated, giving as a result an enrichment of Fe and Mn oxyhydroxides. Another portion can also diffuse downwards and precipitate as sulfides (amorphous FeS, mackinawite, greigite, pyrite). During these dissolution/precipitation processes, elements normally associated with the oxide phase will also be solubilized and, eventually, they will coprecipitate with the Fe and Mn oxyhydroxides or with the Fe sulfides. Although this enrichment process is typical of normal marine sediments, there are other sedimentary environments where the accumulation of trace metals in iron sulfides can be accomplished via different processes. For example, in hydrothermal systems, thermochemical sulfate reduction (defined as the abiotic process whereby SO_4^{2-} is reduced to H_2S without catalysis by microorganisms; Elsgaard et al., 1994) becomes more important than microbially-mediated sulfate reduction. Hence, a difference in the type of sulfate reduction involved may change the degree of association of trace metals with iron sulfides. Pyrite is considered one of the major authigenic minerals found in anoxic-sulfidic sediments (Berner, 1981) and can be a very important reservoir for a number of trace elements (*e.g.*, As, Cd, Co, Cu, Hg, Mo, Ni, Pb, Zn; Huerta-Diaz and Morse, 1992). In this study we present the results of chemicals extractions of sedimentary pyrite and associated trace elements from a 39-cm long sediment core colected in Guaymas Basin in the Gulf of California, an active sediment-covered hydrothermal system located in a seafloor-spreading center underlaid by productive-rich surface waters.

2. Materials and Methods

Sediment samples were subjected to the extraction procedure developed by Huerta-Diaz and Morse (1990) which obtains the following three operationally-defined fractions: (1) HCl, obtained after digestion of a sediment sample with 1M HCl (comprises Fe monosulfides, amorphous and crystalline Fe, Mn oxyhydroxides, carbonates, and hydrous aluminosilicates); (2) Silicate, extracted with 10M HF (comprises mainly clays); and (3) Pyrite, obtained after digestion with concentrated nitric acid (comprises pyrite and associated trace metals).

3. Results and Discussion

Sediments from Guaymas Basin show concentrations of pyrite-Fe (maximum of 129 μmol/g dry wt) and pyrite-Me similar to concentrations measured in other anoxic-sulfidic sediments (*e.g.*, Baffin Bay, Texas and Green Canyon in the Gulf of Mexico; Huerta-Diaz and Morse, 1992). However, our results also indicate that the largest concentrations of trace metals are found in the pyrite fraction, rather than in the HCl fraction, except in the case of Cd and Mn, where the opposite trend is found. The difference between the sediments of Guaymas Basin and other

anoxic-sulfidic sediments lies in the degree of pyritization (DOP), a term developed by Berner (1970), in which the extent of Fe pyritization is evaluated using the equation

DOP = (Pyrite-Fe)/[(Pyrite-Fe) + (HCl-Fe)].

Where pyrite-Fe and HCl-Fe correspond to the iron concentrations in the pyrite and HCl fractions, respectively. Similarly to this term, the concept of degree of trace metal pyritization (DTMP) was developed by Huerta-Diaz and Morse (1990, 1992) to evaluate the extent of association of a given trace metal (Me) with pyrite, a term defined as

DTMP = (Pyrite-Me)/[(Pyrite-Me) + (HCl-Me)].

Where pyrite-Me and HCl-Me correspond to the metal concentrations in the pyrite and HCl fractions, respectively. Application of these two equations to our results indicate that Fe is highly pyritized (90-100%) and, contrary to what has been found in other anoxic sulfidic sediments, Guaymas Basin show high degrees of trace metal pyritization for all the trace metals considered, as shown in the following table:

Table 1: Range of degree of trace metal pyritization of samples from Guaymas Basin.

DTMP (%)	**Co**	**Cr**	**Cu**	**Ni**	**Zn**	**Cd**	**Pb**	**Mn**
Maximum	100	100	100	100	100	90	81	27
Minimum	61	100	100	65	100	14	59	2.5

4. Conclusions

The hydrothermal sediments from Guaymas Basin present concentrations of pyrite and associated trace metals similar to the ones found in anoxic-sulfidic sediments. However, the values of DOP and DTMP measured in the hydrothermal sediments are considerably higher than the corresponding values measured in anoxic-sulfidic sediments, suggesting that thermochemical sulfate reduction is a process more effective for the pyritization of iron and trace metals.

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SOME PROBLEMS FOR THE SPECIATION OF HEAVY METALS IN MARINE SEDIMENTS.

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1. Introduction

Extraction tests are commonly used to study the mobility of metals in soils and sediments by simulating different environmental conditions or severe changes on them (KERSTEN and FÖRSTNER, 1986; KHEBONIAN and BAUER, 1987; KENNEDY et al., 1997).
The results obtained by determining the extractable elements are dependent on the extraction procedure applied. In this paper, two types of extraction are considered : single and sequential, and two kinds of sediments are evaluated: calcareous and non calcareous.

2. Materials and Methods

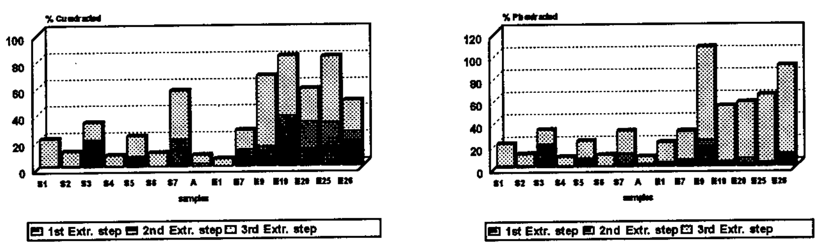
Twelve sediments (calcareous and non calcareous) were treated both by the selective extractants approach (sodium dithionite + sodium citrate (MEHRA and JACKSON, 1960), DTPA (LINDSAY and NORVELL, 1978), HNO_3 0.1M and Na HCO_3 0.1N (OLSEN and SOMMERS, 1982)) and by the sequential extractions procedure (URE et al, 1993). The metals studied were lead, cadmium, zinc and copper. The total metal content was by flame atomic absorption spectrometry (FAAS) and electrothermal atomic absorption spectroscopy (ETAAS). For this purpose, the samples were slurried in a dilute hydrofluoric acid solution and the suspensions were directly introduced into the flame by means of a simple flow injection manifold (BAUTISTA et al, 1994).
X-ray diffraction analysis and scanning electron microscopy with energy dispersive X-ray analysis (SEM-EDX) were used to evaluate the single extractions procedure and the three steps involved in the sequential procedure.

3. Results and Discussion

X- ray diffraction and SEM- EDX were performed on the non-treated particulates and on the residues after the attack. The results enabled us to put forward some interesting speciation hypotheses, that would have been certainly different, and probably erroneous if based solely on an analysis of the extracts.
The different mineralogy is responsible for the results. The quantity of metal analysed in a sample was related with its mineralogy. In the calcareous sediment some heavy metals were mostly bound to calcite and dolomite, and different results were obtained depending on the proportion of these carbonate minerals.
Our results show that calcareous sediments show a peculiar behaviour due to the high amount of carbonates present (MARTINEZ-SANCHEZ et al, 1996). For this reason there was no correlation between the results obtained by sequential extractions and those found by means of selective extractants. The methodology usually recommended for mobilization and speciation studies is not suitable when dealing with this particular type of sediment.
When non calcareous marine sediments were studied by means of the same approaches the results also depended on the experimental procedure used. Our data indicate that the relative proportions of piroxenes, Fe- oxides, and clay minerals are very important to obtain a reliable speciation scheme of the heavy metals.

Figure 1 shows the results for lead and copper obtained by a sequential extraction method.

Figure 1
Sequential Extraction



4. Conclusions

The speciation of heavy metals (Pb, Zn, Cu and Cd) in sediments poses methodological problems which are especially severe when dealing with highly calcareous sediments. The use of selective extractants provides a more realistic way of studying the risk of heavy metal mobilization in such type of samples, since environmental conditions can be simulated if suitable extractants are selected. To obtain reliable speciation data when dealing with this type of sediment, which is abundant in the Mediterraneam Basin, the commonly accepted and recommended sequential extraction methodology needs to be revised.

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SUCCESSION OF LAKE PHYTOPLANKTON AND FUNGI COMMUNITIES UNDER THE EFFECT OF TOXIC METALS

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1. Introduction

Investigations of the phytoplankton and water fungi of Lake Ladoga from 1956 to 1990 have shown the rapid eutrophication process. Changes in plankton communities of Lake Ladoga associated with eutrophication, represented a typical succession of this kind. There is also the industrial pollution in Lake Ladoga in addition to eutrophication process. Toxic metals constitute an important fraction of chemical pollution in the lake. The great diversity of algal and fungi plankton composition suggests varied responses of organisms in this situation. At the first stage of experimental investigations the facts of typical forms of plankton succession are coming to light. Our paper discusses the changes in plankton community structure due to increasing toxic metals ions contents in water.

2. Materials and Methods

The purpose of the study was to estimate the response of plankton communities and individual algae and fungi species in the presence of high content of toxic ions in lake warers. The investigations were carried out on the basis of natural plankton of Impilahty Bay (northern part of Lake Ladoga) in August 1994-1997. One part of the observations was performed in May 1995-1997 in the outlet Neva River. Surface water was utilised to test the response of plankton fungi. The phytoplankton net samples from surface level of lake water were used for the experiments with algae. The impact of **Cd**, **Cr**, **Hg**, **V**, **Pb**, **Ce**, **Cu**, **Zn**, **Ni** and **Co**, on the natural communities of algae and fungi was estimated.

The phytoplankton net samples were placed into the 10-ml experimental bottles. To identify water fungi response, the traditional microbiological methods with addition of heavy metals to agar plates were used. Toxic concentrations (from 0.1 to 50 mg/l) were used depending on different metals. The total volume of the solution of metal should be no more than 0.5 ml. After 24 hours of exposure in plankton samples the ratio of dead to alive cells was determined using the fluorescent microscopy. The Petri (agar) plates with the fungi cultures have been incubated during 21 days. The results have been accounted upon a 3-point system (good growth, weak growth, no growth). A morphology of cells was watched on the liquid media. An amount of biomass was accounted by the weight method in absolutely dry grams per 1 liter (ADG/l) of medium. All experiments were accompanied by determination of water temperature and hydrochemical parameters in the lake.

In all 49 series of experiments were made with plankton algae and 14 series with fungi of Lake Ladoga.

4. Results

The phytoplankton of Neva outlet in May was typical for the spring pulse of Lake Ladoga one. The diatom algae *Aulacosira islandica (O.Mull.) Sim*. Dominated in our samples, with addition of green *Sphaerocystis schroeteri Chod.* and yellow-green *Tribonema affine West.*

In August 1994-1997 the phytoplankton communities of Impilahty Bay were typical so as for the late summer in Lake Ladoga. A diversity of algae species was very high. The prevailing groups were *Bacillariophyta, Cyanophyta,* and *Chlorophyta* with addition of *Tribonema affine (Xanthophyta).* In this period there were about 24-44 species and forms of algae in plankton samples.

The dominant fungi were represented by genera *Saprolegnia, Penicillium, Trichoderma.* In total, 17 species related to 9 genera have been distinguished. A reaction of organisms to the presence of xenobiotics in medium was determined using an indicator of mass character, the change of the growth speed, and increase of yeast. During experiments, it was found that g. *Trichosporon* had inhibitor effect

reacting with **Ce**. With different concentrations of **Cr** and **Ce**, an indicator of the mass character for all yeast, besides representatives of genera *Candida* and *Debaryomyces*, varied insignificantly.

Table 1. The impact of toxic metals on the plankton algae and water fungi.

Species	Toxic metals	Middle-toxic metals	Low-toxic metals
Algae			
Asterionella formosa	Cu, Zn, Ni	Cd, Pb	V, Cr, Hg, Ce, Co
Aulacosira ambigua	-	Cd, Cr, Hg, Pb, Cu, Zn, Ni, Co	Ce
Aulacosira islandica	V	-	Hg, Ce, Pb
Diatoma elongatum	Cu, Zn, Co	Cr, Ni	Cd, Hg, Pb, Ce
Fragilaria crotonensis	Cu	Cd, Pb, Zn, Ni, Co	Cr, Hg, Ce,V
Tabellaria fenestrata	-	Cd, Cu, Zn, Ni, Co	Cr, Hg, Pb, Ce
Anabaena flosaquae	Cd, Cr, Hg, Pb, Zn, Co	Ni	Ce, Cu
Anabaena spiroides f.f.	Cd, Cr, Hg, Pb, Co	Ni	Ce, Cu
Oscillatoria planctonica	Cd, Hg	Pb, Ce	Cu
Oscillatoria tenuis	Hg, Zn	Cd, Cr, Pb, Ni	Ce, Co, Cu
Woronihinia naegeliana	Hg, Zn	Ni, Cr	Ce, Co, Pb
Dictiosphaerium pulhellum	-	Cr, Ni, Co	Hg
Eudorina elegans	Cu	Cd, Cr, Ce, Zn, Ni, Co	Hg, Pb
Pediastrum duplex	-	Cr, Zn, Ni, Co	Cd, Hg, Pb, Ce, Cu
Sphaerocystis schroeteri	Cu	Cr, Zn, Ni, Co, V	Hg, Pb, Ce
Staurastrum gracile	Cu	Pb, Ce, Ni, Co	Cd, Cr
Tetraspora tenera	Zn	Hg, Pb	-
Tribonema affine	Cu	Cd, Pb, Co	Cr, Hg, Ce, Zn, Ni, V
Ceratium hirundinella	Ce, Cu	Cd, Co	Pb, Zn, Ni, Hg
Fungi			
Trichosporon sp.	Ce	Hg, Pd, Cr	Co, Ni, Cu, Zn, V
Debaryomyces cantarelli	-	Hg, Ce, Cr	Cu, Zn, Ni
Candida krusei	-	Ce, Cr	Co, Ni, V

For mentioned genera it changed for 50 and more percent. In experiments with a toxic effect on yeast was not found. All species were tolerant to the studied concentrations. With maximum concentrations of metals studied, the biomass increase was less for *Candida krusei* and *Debaryomyces cantarelli*. As a result, the toxicity of metals, used in experiments, can be classed into three sections for individual species of algae and fungi (toxic, middle-toxic, low-toxic) (Table1).

4. Conclusions

Changes in the composition of phytoplankton and water fungi's assemblages associated with toxicity of metals represented some typical forms of succession. A comparison shows that trends of successions are similar within different seasons. This means that the impact of metals inevitably produces the reconstruction of food chains and the energy flow in the lake ecosystem. To take an example, the toxicity due to **Hg, Pb** and **Cd** suppressed blue-green algae dominants in summer plankton. In response to the increase of toxic pressure the primary production decrease. On the other hand, the initiation of phytoplankton communities composed by one or two very productive species is possible. Once the plankton with dominance of *Tribonema affine* occurs, it may be very productive in Lake Ladoga.

During experiments, it was determined that gg. *Candida krusei* and *Debaryomyces cantarelli* are the most sensitive species within the yeast community of Impilahty Bay, that was characterized by the change of mass character indicator, speed and character of growth, and morphologic changes of cells.

TOTAL AND DISSOLVED ELEMENTS IN SUPERFICIAL WATERS OF THE ANTARCTIC PENINSULA

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1. Introduction

The material in the hydrosphere is in three main phases: dissolved material, particulate material suspended in water, and deposited sediments. In Antarctic peninsula ponds formed by fusion of freshly deposited snow and also by glacier fusion, can change their size and shape from one year to another. This study intends to quantify the elements in superficial waters (ponds) of the Antarctic peninsula and determine their possible origins.

2. Materials and Methods

Triplicate superficial water samples were taken from six lakes at King George Island, Shetland Islands, Antarctic Peninsula, and stored in plastic bottles previously rinsed with diluted nitric acid, during the 1997 and 1998 Chilean Antarctic Expeditions. One fraction of each sample was filtered through 0.45 μm filter membrane (dissolved fraction); another was adjusted to pH 2 adding nitric acid and dispersed by 1-h ultrasonic treatment before filtration (total concentration). Aluminum, Cr, Ni, Cu, Zn, Se, Cd, Sb and Ba were quantified using ICP/MS.

3. Results and Discussion

The levels of heavy metals in waters, have been arbitrarily divided into two components: filterable ($\leq$ 0,45 μm), called dissolved fraction, and suspended matter, which eventually produces sediments (Khalid, 1980). A comparison of total and dissolved mean element concentrations found in Antarctic waters is given in Table1. We should note the differences in each case for Al, Ni, Cu Zn, and Ba. Figure 1(a and b) shows that the concentrations of dissolved elements are different: a) depending on the pond, e.g. Zn and Ba varied strongly, and b) from one year to another, e.g. from 1997 to 1998 Al and Cu always while Se increases. These results could be reflecting the influence of elements coming from the atmosphere; as aerosols or rain produced locally or traveling large distances, or coming from the sediments removed by the water being continually moved by the wind at the edges of the ponds. To support this theory, the results obtained for atmospheric aerosols, rain and soils are considered (Préndez and Muñoz, 1997).

4. Conclusions

Some elements mainly heavy metals are adsorbed by particles $\leq$ 0,45 μm which means that in case of using these waters for drinking, analysis should include the suspended particles (total concentrations).

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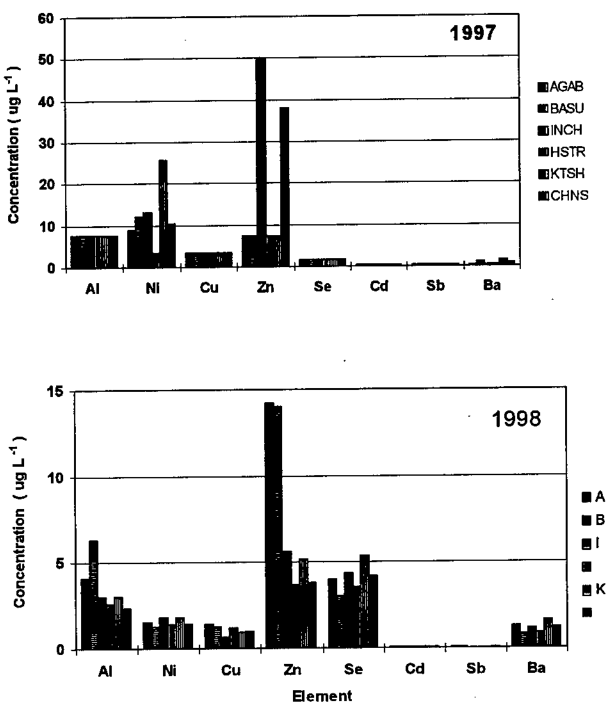

Figure 1. Dissolved mean element concentrations in Antarctic waters for years 1997 and 1998

Table 1. Total and dissolved mean elements concentrations in Antarctic waters

Pond	Content	Al	Cr	Ni	Cu	Zn	Se	Cd	Sb	Ba
						ppb				
AGAB	Dissolved	bld[1]	3,0	bld	bld	bld	3	bld	bld	1,3
	Total	15	2,9	bld	bld	17	4	bld	bld	1,2
BASU	Dissolved	6	1,3	bld	bld	5	bld	bld	0,05	1,1
	Total	30	1,7	23	10	105	bld	3,15	0,07	40,7
INCH	Dissolved	bld	1,6	bld	bld	bld	2	bld	bld	0,9
	Total	24	1,8	5	20	150	1	0,6	0,02	42,4
HSTR	Dissolved	2	2,2	bld	bld	bld	1	bld	0,02	0,4
	Total	14	2,0	bld	6	61	3	bld	0,03	14,4
KTSH	Dissolved	bld	2,6	bld	bld	7	2	bld	bld	1,2
	Total	18	3,0	bld	5	33	3	bld	bld	0,5
CHNS	Dissolved	bld	2,0	bld	bld	bld	2	bld	0,07	0,7
	Total	23	2,3	5	9	16	2	0,05	0,11	1,7

[1] bld = below limit of detection.

TRACE METAL STUDY OF THREE MAJOR NORTH-WESTERN ARCTIC ESTUARIES

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1. Introduction

The majority of river suspended material (70-95%) is deposited on the river-sea geochemical barrier, huge sedimentary bodies are formed here, catching elements contained in river suspensions (Artemyev, 1996; Postma, 1980). A considerable amount of trace metals, which come from human polluting activities or natural sources, enter into the marine environment from rivers and affect the estuarine systems where they are quite often deposited. In the present work, the trace metal content in surface sediments of three major Western Russian Arctic estuaries is discussed in relation to suspended particulate matter (SPM) composition.

2. Materials and Methods

During the scientific cruise to the White and Barents seas (June-July, 1995) on board of MS "Professor Vladimir Kuznetsov" in the framework of INTAS project 94-391 "Biodiversity and Adaptation Strategies of Arctic Coastal Marine Benthos" the hydrological and geochemical characteristics of Northern Dwina, Mezen and Pechora estuaries were determined. Salinity and temperature vertical depth profiles at several sampling stations along the salinity gradients of estuaries were obtained by CTD probe. SPM samples were collected by filtration on Millipore, cellulose filters (47mm/0,45μm), surface sediment samples were collected by Van Veen grab. Concentrations of SPM in water were calculated as difference in weight of dry filters with material and preweighted clean filters.

In the stationary laboratory SPM samples on filters as well as fine-grained surface sediment fraction (<63 μm) were separated by sieving from upper (0-1 cm) sediment layer were subjected to total acidic dissolution with further determination of Zn, Co, Cu, Ni, Pb, Mn, Fe and Al contents by ICP AES method (Totland et al, 1992).

3. Results and Discussions

Results of metal contents in sediments and SPM of three estuaries are presented in table 1 and 2.

Table 1: Average total metal contents in surface fine grained sediments of Northern Dwina, Mezen and Pechora estuaries

Estuary	Al (%)	Fe (%)	Mn (ppm)	Zn (ppm)	Pb (ppm)	Co (ppm)	Cu (ppm)	Ni (ppm)
N.Dwina	5,6	3,71	1504	72	39	21	17	37
Mezen	4,8	2,1	532	60	11	16	6	23
Pechora	5,2	2,6	1021	71	22	20	11	39

Table 2: Average total metal contents in SPM of Northern Dwina, Mezen and Pechora estuaries

Estuary	**Al** (%)	**Fe** (%)	**Mn** (ppm)	**Zn** (ppm)	**Pb** (ppm)	**Co** (ppm)	**Cu** (ppm)	**Ni** (ppm)
N.Dwina	0,92	0,8	626	72	47	7	94	23
Mezen	2,4	1,6	365	200	44	11	50	20
Pechora	3,4	2,8	677	165	59	16	110	30

The SPM of three estuaries were enriched by an average factor of 8 times in Cu with respect of those in surface sediments. Under estuarine conditions Cu is probably associated with the most labile fraction of suspended organic matter, which is subjected to microbiological destruction during sedimentation while Cu is released into solution. The contents of Zn and Pb are also elevated in SPM relative to surface sediments, average enrichment factor is 2,2 for Zn and 2,6 for Pb, that may indicate their significant affinity to labile organic components of SPM. For Ni, Co, Fe and Al average enrichment factors were >1 within the range 0,4-0,6. These elements showed a strong affinity to mineral components and most resistant organic compounds of suspended material which are accumulated in sediment phase.
The sediments of Northern Dwina estuary are enriched in metals comparing to Pechora and especially to Mezen, while the SPM of Northern Dwina exhibit lower contents of major and some trace metals in relation to the SPM of Pechora and Mezen. This can be caused by specific sedimentation conditions in the Northern Dwina estuary that cause a rapid removal of river material and its accumulation in sediments.

4. Conclusions Trace metal behavior in estuaries is controlled by their different affinity to organic and mineral components of SPM and sediments. The differences in spatial distribution of trace metals in the three investigated estuaries are due to the different hydrological and hydrodynamic features of each estuary, reflected in salinity and temperature distribution.

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ACCUMULATION OF BIOGEOCHEMICAL POLLUTANT IN THE FLOODPLAIN SEDIMENTS OF YAMUNA RIVER (TRIBUTARY OF GANGA) DURING RECENT PAST.

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1. Introduction

In the present study core sediments from the floodplain of the river Yamuna (largest tributary of the river Gangs, India), originate from Yamuntori Glacier in the Mussorie range of the lower Himalayas.

2. Materials and Methods

These core samples were collected by manual drilling from the floodplain of Yamuna Basin at five sampling stations: Saharanpur (next to Himalayas), Delhi, Jagmanpur, Hamirpur, and Allahabad. The core sediments were analysed for metals (Pb, Zn, Fe, Cu, Ni, Cr, Ti, Ba, and Al) and Phosphorus using XRF techniques. Carbon and Sulphur by using Leco Carbon and Sulphur analyser respectively.

3. Results and Discussion

All the studied metals show higher concentration in the upper part of the core, except Jagmanpur core sediments. The higher levels are due to the increase of industrialisation and urbanisation in the recent past. The Jagmanpur core does not follow this the trend as a result of turbulent mixing of sediments from a large number of small streams. All the metals at Delhi region reported higher values in comparison to the down stream cores (except Cr) because Delhi is the most highly industrialised area in the entire basin. The sediment enrichment factor of all the studied metal showed highest value in the floodplain of Delhi regions because the Industrial effluents - carried by drainage channels from the catchment area contain large amount of metals and deposited with the floodplain sediments.

Phosphorus concentration ranges from 272 mg/g to 940 mg/g. This is comparable to the range of average content of Phosphorus in the sediments of Indian rivers -11000 mg/g (Subramanian,1993). Delhi sediments reported highest value of Phosphorus 321 - 940 mg/g. Carbon and Sulphur values are higher in younger strata (upper) then older strata of floodplain. It signifies the change in nutrition dynamic of basin in recent past as a result of anthropogenic activity. The Sharanpur area has highest rate of sedimentation (5.99cm/yr). The rate of sedimentation values with both techniques, ^{137}Cs and ^{210}Pb are reasonably similar.

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Bioavailability of Trace Elements

(Technical Session 5)

FIELD TEST OF AMENDMENTS TO REDUCE THE IN SITU AVAILABILITY OF SOIL LEAD

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1. Introduction

Pb contamination of soils is viewed as a major environmental problem, primarily due to the human health effects associated with direct ingestion of Pb contaminated soils. There has been a growing research emphasis on remediating Pb contamination in situ. The basis for this approach has been that certain Pb minerals are much less soluble than others and therefore should have lower bioaccessibility. By altering the mineral form of Pb in soil, the bioaccessibility of Pb should also be reduced. A field study was set up, with the cooperation of the US EPA Remediation Technologies Development Forum (RTDF) Inplace Inactivation and Natural Ecosystem Restoration Technologies (IINERT) soil metals group to test the effectiveness of the most promising soil amendments under field conditions. A number of parameters, including plant metal concentration and in vitro bioaccessibility measurements have been used to test the ability of amendments to reduce Pb availability in situ.

2. Materials and Methods - Joplin Field Study

The *in situ* treatability study was installed in Joplin, MO in March, 1997. Joplin was part of the old Pb belt in Missouri and much of the town is currently on Superfund's NPL list. The field site is located on a vacant city lot. SEM analysis of the soil indicated that the majority of Pb in the soil was present as Pb carbonates (Blanchar, University of Missouri). Treatments were installed in 2 x 4 m plots using a completely randomized design with 4 replicates. Treatments were weighed on a per plot basis and then tilled into the soil to a 12.5 cm depth. A list of treatments is included in Table 1. "Iron Rich", a high Fe by-product from titanium processing, was supplied by DuPont Chemical. The compost was a high Fe product using lime-stabilized biosolids as a feedstock from Washington, DC. All materials were added to soil on a dry weight basis. Three months after amendment application, $CaCO_3$ (71% purity)was added to each plot to bring the pH to 7. The amount of lime required ranged from 156 kg per plot (3.2% P TSP) to 38 kg per plot (10% Compost + 0.32% P as TSP). After lime amendment, seed (K31 Tall Fescue) was hand scattered over the surface of the plots.

In September , 1997 soil and plant samples were collected from each of the plots. Plants were analyzed for total metals. Soils were analyzed for pH and *in vitro* Pb extractability using the modified rapid procedure developed by Ruby et al (Table 1). To account for the differences in total soil Pb, statistics were performed on the ratio of plant or bioaccessible Pb to total Pb for each plot. Data was log transformed to reduce variance. Results presented include the actual value for each variable as well as the negative log of each ratio.

Table 1. Soil and plant lead and *in vitro* (at pH 2.2) lead for soil and plant samples collected from the field site in September, 1997. The ratio variables indicate the relative uptake and bioaccessiblity of lead to total soil lead. Soil pH ranged from 6.45 in the control treatment to 7.60 in the 10% compost treatment

Treatment	Soil Pb	Plant Pb	p(plant Pb/soil Pb)	*In vitro* Pb	p(*in vitro* Pb/soil Pb)
	----------	---------- mg kg^{-1}	----------	----------	
Control	2892 ± 680	23.3 ± 5.62	4.87 ab	20.7 ± 4.21	4.09 a
1% P	4056 ± 280	24.6 ± 6.08	5.20 abcd	31.1 ±5.92	4.92 a
3.2%	3340 ± 568	4.54 ± 1.90	6.76 e	13.4 ±2.84	5.53 d
1% Fe+ 1% P	3660 ± 801	25.1 ± 6.25	5.03 abc	21.8 ±6.38	5.18 abc
2.5% Fe+ 0.32% P	2389 ± 827	7.70 ± 2.17	5.74 d	12.8 ±5.35	5.32 cd
2.5% Fe + 1%P	1861 ± 492	16.0 ± 4.67	4.78 a	10.3 ±3.82	5.27 bcd
1% P as Rock Phosphate	2108 ± 433	11.9 ± 6.43	5.56 cd	14.4 ±3.19	4.99 ab
Compost at 10%	2617 ± 560	12.7 ± 5.97	5.57 cd	15.8 ±4.47	5.16 abc
10% Compost + 0.32% P	1517 ± 507	6.78 ± 2.71	5.47 bcd	8.14 ±3.19	5.27 bcd
10% Compost + 1% P	2965 ± 809	15.7 ± 4.08	5.21 abcd	16.5 ±5.47	5.33 cd

3. Results and Discussion

Several amendments significantly reduced the availability of soil Pb as measured by both plant Pb concentrations and in vitro accessible Pb with addition of 3.2% P as the most effective amendment. Significant reductions in Pb availability were also seen in the rock phosphate amended soils as well as in some of the compost amended and Iron Rich amended soil treatments. Addition of 1% P as TSP did not have any effect on Pb availability relative to the control. This treatment has shown highly significant reductions in Pb availability under controlled conditions. It is possible that the absence of any reduction in Pb availability in the field trial may be related to the intermediate changes in soil pH associated with the 1% P addition. This amendment may have reduced pH sufficiently to increase Pb solubility and reprecipitation as intermediate, relatively soluble Pb orthophosphates rather than as highly insoluble chloropyromorphites). Addition of P as rock phosphate was not associated with a change in soil pH. This amendment may have altered the mineral form of the more soluble Pb fraction without dissolving a large fraction of the larger size Pb carbonate precipitates.

THE DIFFERENCES AMONG PLANT SPECIES TO ACCESS NUTRIENT AND TRACE METAL ELEMENTS IN SOILS DETERMINED USING A RADIOACTIVE ISOTOPE DILUTION METHOD

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It is commonly known that different plant species vary in their ability to access nutrients and contaminant elements in soil. It is also known that metals are fixed or adsorbed to a variety of soil surfaces with differing capacities to bind strongly. The objective of this study is to identify differences among plant species to access Cd, Zn and P (and Pb) in a range of Australian soil types, using the radioisotope dilution ('L-value') method. This method will allow us to determine whether plants are taking up elements from the exchangeable pool, identified by isotopically exchanged elements, or other 'fixed' pools. The study will show the role of growing plants (rhizosphere effect) in modifying uptake and also the soil characteristics that influence nutrient and contaminant element availability.

Agricultural soils from Queensland (high Fe oxide concentration) and South Australia (high allophane and high clay content) and contaminated soils from home gardens close to a Pb smelter, from contaminated 'fill' sites and urban/industrial soils from Sydney, will be used. The soils will be treated with 32P, 65Zn and 109Cd, incubated for two weeks at 25° C and 40°C and then planted with wheat (*Triticum aestivum* Var. Krichauff) canola (*Brassica napus* var. Hyola 42) and white lupin (*Lupinus albus.* Var. Ultra). Higher temperatures have been shown to enhance fixation reactions.

This study will elucidate some of the mechanisms involved in trace metal availability in a range of soil types: relatively 'clean' agricultural soils and soils contaminated with a range of low to high trace metal concentrations.

THE ROLE OF BASAL ROOTS IN THE SUPPLY OF CADMIUM TO THE TUBER OF POTATO (SOLANUM TUBEROSUM L.)

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1. Introduction

The potato (Solanum tuberosum L.) has a highly branched, fibrous root system which is made up of four different types of roots, as described by Kratzke and Palta (1985). The roots have been shown to have different roles in terms of providing water and nutrients to the tuber of potato. The basal and junction roots, which originate from the base of the stem and the junction between the main stem and the stolons respectively, have been shown to provide water primarily to the aerial parts of the plant and have little importance in the water supply of the tuber (Kratzke and Palta 1985). Water is provided to the tuber via xylem flow from small roots originating directly from the stolon (stolon roots) and the tuber (tuber roots). These roots also play an important role in the supply of calcium (Ca) to the tuber, with the stolon roots providing an estimated 60% of the Ca in the tuber (Kratzke and Palta 1986).

The pathway of cadmium (Cd) uptake into the potato tuber is unknown but its high mobility in the xylem sap suggests that the stolon and tuber roots may be important in the transport of Cd to the tuber. Cadmium may also enter the tuber via direct transport across the periderm. A split-pot method was used to investigate the role of the different types of roots in supplying Cd to the tuber.

2. Materials and Methods

Seed tubers of cultivar Kennebec were grown in horizontally divided pots in 15 litres of a 3:4 mix of peat moss and sand. Prior to potting, half of the peatmoss/sand mix was labelled with ^{109}Cd to a specific activity of 4 $kBq\ kg^{-1}$.

The experiment consisted of three treatments; 1) supply of ^{109}Cd to the basal roots by placing labelled soil in the bottom section of the pot (basal root zone), 2) supply of ^{109}Cd to the stolons and tubers directly by placing labelled soil in the top section of the pot (tuber zone) and, 3) supply of ^{109}Cd to the basal root, stolons and tubers by placing labelled soil in both sections of the pot (whole root system). Each treatment was replicated four times.

The bottom section of each pot was filled with 10 kg of the soil mix (labelled or unlabelled, depending on treatment allocation) and the seed tubers planted just below the surface. Two weeks after planting, a second pot, with a hole in the base, was placed on top of the soil so that the stem of the tuber was threaded through the hole. All the basal roots remained in the bottom section of the pot, while any initiating stolons were in the top section of the pot. The hole was then sealed to ensure that there was no leakage between the two compartments. The top section of each pot was filled with 5 kg of either labelled or unlabelled soil mix.

Harvest occurred 10 weeks after emergence. Plants were divided into new leaves (first 4 leaves from the growing apex), old leaves, stems and tubers and the concentration of Cd and ^{109}Cd in each component was determined.

3. Results and Discussion

^{109}Cd supplied to the basal root zone was found to account for 80–85% of the Cd in the tuber. This suggests that the majority of Cd in the tuber is supplied by translocation from the leaves, via the phloem. Uptake of ^{109}Cd from the tuber zone was less than the basal root zone but still important, providing 15–20% of Cd in the tuber. The development of fine stolon roots in the tuber zone was extensive. Transport is likely to have occurred via a xylem connection between these roots and the tuber, although, direct uptake of ^{109}Cd across the periderm of the tuber can not be discounted.

As expected, the majority of Cd in the above ground material was supplied by the basal root system, with a

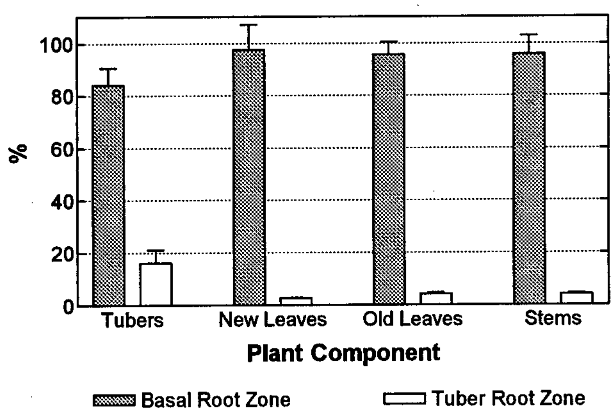

Figure 1: **Percent of Cd in plant components supplied by the basal or tuber root zone**

small fraction, 2–4%, originating from the tuber zone. This supports findings by Kratzke and Palta (1985) of a continuous xylem connection between the stolon roots and the upper portion of the plant.

4. Conclusions

Cd appears to have a high mobility in the phloem, with the major pathway of uptake being from the basal roots to the leaves, most likely in the xylem, followed by translocation to the tubers via the phloem sap. In addition, this study showed that stolon and tuber roots also play an important role in the supply of Cd to the tuber, possibly via a direct xylem connection from these roots to the tuber.

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CHEMICAL MOBILIZATION AND ACQUISITION OF CADMIUM AND COPPER BY LUPINUS ALBUS L. AND TRITICUM AESTIVUM L.

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1. Introduction

Several plant species are known to increase the efflux of di- and tricarboxylic acids as a result of phosphate (P) deficiency, for example white lupin (Dinkelaker et al., 1994) and red clover (Gerke and Meyer, 1995). The organic anions increase to the solubility of heavy metals in soil (Chairidchay and Ritchie, 1990; Gerke, 1995).

We, therefore, investigated the effect of carboxylate efflux by white lupin on the acquisition of copper (Cu) and Cadmium (Cd) and compared the results with the non- mobilizing wheat.

2. Materials and Methods

Lupinus albus L. (cv. Lublanc) and Triticum aestivum L. (cv. Star) were cultivated in 3 kg pots in a growth chamber for 73 and 66 days, respectively. The cultivated soil was a humic podzol, with concentrations of aqua regia- extractable metals of 0.55 mg Cd and 4.9 mg Cu/kg soil. The treatments included foliar P application every second day, in order to white lupin and the application of 2 mg Cd/kg soil in one half of the pots. Soil solution of the cluster root regions of white lupin (proteoid roots) were recovered according to Gerke et al. (1994). Organic acid concentrations were determined by SPE/HPLC, as described by Gerke (1995), metals by GFAAS.

3. Results

In the rhizosphere soil solution of wheat and in the bulk soil, we detected no organic acids by HPLC. In the rhizosphere soil solution of proteoid roots of white lupin we determined high concentrations of citrate and some malate (Table 1).

The proteoid root induced citrate excretion increases the Cu solubility in the rhizosphere but not that of Cd.

In agreement with the results we found that the Cu- influx of white lupin was around five times higher than that of wheat (Table 2). The Cd- influx was higher in wheat than in white lupin especcially in the Cd treated soil.

Table 1. Organic acid concentrations in the proteoid rhizosphere of white lupin and in soil solution without plant depending on the application of Cd.

	Proteoid rhizosphere				Reference solution	
	Citrate µM	Malate	Cd μgL^{-1}	Cu	Cd μg^{-1}	Cu
treatment						
- Cd	830	88	3.1	30.3	2.1	11.7
+ Cd	1024	73	22.8	29.8	25.8	9.1

Table 2. Influx of copper and cadmium in white lupin and wheat

	Cd [mol 10^{-18} s^{-1} cm^{-1}]		Cu [mol 10^{-18} s^{-1} cm^{-1}]	
	White lupin	Wheat	White lupin	Wheat
treatment				
-P -Cd	0.20	0.25	17.4	3.2
-P +Cd	1.72	2.15	14.5	3.1
+P -Cd	0.18	0.23	16.2	2.9
+P+Cd	1.83	2.64	17.2	3.6

4. Discussion

In soil, low inavailable P white lupin excretes high quantities of citrate in the proteoid rhizosphere. Citrate strongly mobilizes Cu but not Cd in the soil used.
In accordance with this result the Cu- influx is much higher in white lupin compared to the non-mobilizing species wheat.

A different picture exists for Cd. The influx of Cd is lower in white lupin compared to wheat especially at high soil Cd. We also found a significant increase in ctrate concentrations in the proteoid rhizosphere soil solution induced by the Cd treatment and conclude that the excretion of citrate may help to exclude Cd from the lupin plants. The biochemical mechanisms for Cd exclusion is unknown, however Cd- species calculations in the soil solution will clarify whether Cd- citrate complexes contribute to Cd in the soil solution.

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EFFECT OF PHOSPHORUS AND OTHER SOIL AMENDMENTS ON SOIL LEAD, CADMIUM, AND ZINC BIOAVAILABILITY.

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1. Introduction

Lead contaminated soil is a primary source of Pb exposure to young children. Phosphorus amendment is being considered as an alternative to soil excavation (EPA 1996). It is reasonable to believe that P-amended Pb-contaminated soils would cause less of an increase in blood Pb concentrations upon ingestion by children than the unamended soils, due to the formation of stable Pb phosphates (pyromorphites) in the presence of P. However, there are some unresolved issues related to this technique such as long term stability of the newly formed pyromorphites; the influence of P source and rate; and the effects of P on other metals commonly associated with Pb in contaminated soils (e.g., Cd and Zn). The purpose of this study was to evaluate the influence of P amendment on five contaminated soils/ mine wastes. Data will be presented on the effect of time, P rate, P source, and preacidification on soil Pb, Cd, and Zn bioavailability.

2. Materials and Methods

Five contaminated soils/mine waste materials (TCR, AR, USDA, Dearing, and Galena) were collected from the Tri-state mining area. Total metal concentrations ranged from 1200 to 9100 mg Pb/kg, 30 to 190 mg Cd/kg, and 4500 to 42600 mg Zn/kg. Seven treatments were used: zero P (control); 2500 mg P/kg as triple super phosphate (TSP2500), phosphate rock (PR2500), acetic acid followed by TSP (acetic), and phosphoric acid (H_3PO_4); and 5000 mg P/kg as TSP (TSP5000) or PR (PR5000). Predetermined amounts of CaO were added for all samples, except for the control and PR, 24 hrs after P treatment to increase the soil pH. Triplicate samples were incubated for seven different sampling times (3days, and 1, 4, 12, 24, 36, and 48 weeks) at 20% gravimetric moisture content and 25°C. Air dried samples were analyzed for soil pH and bioavailable Pb by the *in vitro bioaccessibility* test (Physiologically Based Extraction Test- PBET (Ruby et.al.,). Dissolved concentrations of Pb, Cd, and Zn in PBET extractions were analyzed using ICP-AES. X-ray diffraction data were collected for the <10□m size fraction separated by using an ATM sonic sifter

3. Results and Discussion

The acetic, H_3PO_4, and TSP5000 treatments reduced soil pH down to approximately 5.0 in all five materials used while PR had no effect on soil pH even at the highest rate. Calcium oxide was able to increase soil pH to approximately 7.0. At 3 days and 4 weeks PBET data indicated that P treatments reduced the bioavailable Pb in both stomach and intestinal phases significantly in all five materials used compared to the control except for PR2500 and acetic. Phosphoric acid was the most effective treatment for Dearing with PR being the least effective (Figure 1). For all other materials used PR was equally or more effective than both TSP and H_3PO_4 in reducing bioavailable Pb. Phosphate rock was the most effective in reducing Cd and Zn levels in PBET extractions in most of the materials used.

X-ray diffraction patterns for selected treatments of Dearing are shown in Figure 2. Both 2.85 and 2.97 A hydroxypyromorphite peaks were present in the control indicating that pyromorphite was present in the material prior to P amendment. Addition of P from soluble P sources increased the intensity of 2.85 A peak (marked by an arrow on Figure 2). Phosphoric acid treated samples showed the most intense pyromorphite peak which is in agreement with PBET results.

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Figure 1. Percent bioavailable Pb by PBET, Dearing, 4 weeks

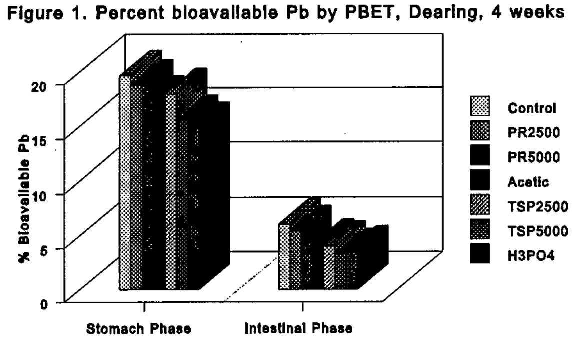
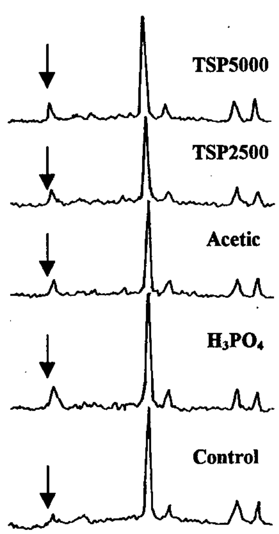


Figure 2. XRD, <10 micron fraction, Dearing, 4 wks.



HEAVY METAL UPTAKE BY LETTUCE (LACTUCA SATIVA L.) FROM DIFFERENT SOILS AND THE RELATIONSHIP BETWEEN MOBILE SOIL HEAVY METALS AND PLANT CONTENTS

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1. Introduction

Various anthropogenic influences (among others metal production and processing, industry, traffic, use of various sprays and fertilizers in agriculture) lead to considerable metal accumulation in soils at some sites. As soil is an ecological sink for pollutants and at the same time habitat of primary producers, an accurate knowledge of heavy metal behaviour in the soil is of immense importance for the safety of all organisms. The easily plant available fraction in the soil solution is of great ecological importance. To predict the mobility of heavy metals several methods using different salt solutions as extractants have been established (Hornburg et al., 1994). For this study 1 M NH_4OAc, 1 M NH_4NO_3 and 0.1 M $CaCl_2$ were investigated as extracting solutions. This paper reports on a part (the year 1997) of a longterm experiment running under field conditions at the Austrian Research Center Seibersdorf since 1987. The aim of this experiment was to examine different soils for their quality, to determine the heavy metal uptake by lettuce, to compare the three extractants concerning their extractability strength, and to relate the mobile fraction to soil properties and to the accumulation of heavy metals in plants.

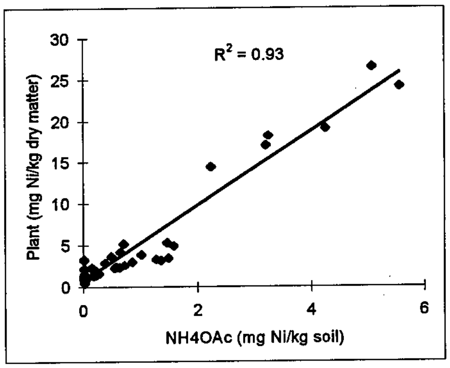
2. Materials and Methods

For this experiment 50 l plastic containers were filled with soils from Untertiefenbach (NÖ), Weyersdorf (NÖ) and Reisenberg (NÖ). The heavy metals cadmium, copper, nickel, vanadium and zinc were added in form of their salts in three concentration levels and three replications (Horak and Kamel, 1990). In 1996 the experiment was extended by two variants with soils from Arnoldstein (Ktn.) and Brixlegg (Tirol) contaminated with lead, zinc, copper and cadmium by smelter emissions. In spring of 1997 lettuce (Lactuca sativa L.) was planted. After harvesting, yield of the plants and heavy metal uptake from the different soils were determined. The individual soils were tested for soil-characteristics such as pH, texture, total content of carbon and nitrogen, organic carbon and carbonate (Blum et al., 1996) as well as for total heavy metals and mobile heavy metal fractions. The total content of heavy metals was extracted by aqua regia and the mobile fraction by three different solutions (1 M NH_4OAc, 1 M NH_4NO_3, 0.1 M $CaCl_2$). The measurements of the heavy metals were performed by AAS and ICP-AES at the Austrian Research Center Seibersdorf.

3. Results and Discussion

The soils showed very different properties. In general, the more acidic and the less clay-containing soils exhibited higher contents of soluble metals. Furthermore, a large difference was observed in the behaviour of the soluble heavy metal fraction between soils treated with heavy metal salts and those contaminated by immission of airborne metals over longtime. The latter displayed a strong

immobilization of heavy metals, therefore plants accumulated relatively less heavy metals than from metal salts treated soils. Lettuce accumulated heavy metals very differently depending on element and on soil. The highest amount of heavy metals was taken from the soil Weyersdorf due to its low metal binding capacity. Cadmium was more accumulated in the leaves, whereas lead, copper and zinc were mostly concentrated in the roots. The strength of extractability was in the order 1 M Ammonium acetate > 1 M Ammonium nitrate > 0.1 M Calcium chloride. However, for cadmium $CaCl_2$ proved to be the strongest extractant. This is likely due to complex formation between chloride and cadmium.



All used extractants proved to be very qualified to establish relationships between the mobile heavy metal fraction and the amount taken up by plants. Best correlation was found for nickel (Fig.1; R^2 = 0,93 – 0,98 for all extractants) and cadmium (R^2 = 0,89 in $CaCl_2$), followed by zinc (R^2 = 0,73 in NH_4OAc). Copper showed no significant correlation with any extractant used.

Fig. 1: Relationship between nickel content in plant and NH_4OAc extractable nickel

4. Conclusions

For all elements the extractants used proved to be suitable for the assessment of plant available fractions of heavy metals in soils, with exception to copper. The extraction order was: 1 M NH_4OAc > 1 M NH_4NO_3 > 0,1 M $CaCl_2$. For cadmium, $CaCl_2$ proved to be a stronger extractant due to complex formation between chloride and cadmium.

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ZINC AVAILABILITY IN CONTAMINATED SOILS AS A FUNCTION OF PLANT (WILLOWS) GROWTH AND ADDITIVE (NH4CL).

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1. Introduction

Phytoextraction of heavy metals from contaminated soils with or without additives is now extensively studied, but little is known about the effect of the plants on the heavy metal pools in the soil. *Salix sp* are known to be able to accumulate heavy metals in their shoots and are thus plant candidates for phytoremediation. The aim of our study was to investigate the effect of *Salix aurita* on the Zn availability in the soil, with and without addition of NH_4Cl to the soil. NH_4Cl is intended to improve plant biomass, decrease pH and help to increase Zn availability to plants. Zinc uptake by *Salix aurita* and Zn distribution within the plants, as well as Zn in the soil extracted by $NaNO_3$, were measured immediately after application of NH_4Cl and after 3 and 8 months.

2. Materials and Methods

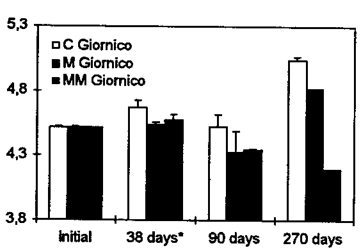
Willow cuttings (*Salix aurita*) were grown for 270 days in greenhouse, in pots containing 2 kg (FW) of three contaminated soils (table 1). After 38 days, a total of 500 ml (100 ml per day) of 0.01M or 0.1M NH_4Cl resp. was added to the pots. Ten-cm long plant samples were cut after 48 days and then every 21 days until the end (270 days). After 90 or 270 days, some plants were divided into leaves, stalks, original stems and roots which were rinsed with EDTA. All plant samples were analysed for Zn. The soil was sampled after 48, 90 and 270 days. The pH, Zn in 0.1M $NaNO_3$ and 2M HNO_3 were followed throughout the experiment.

Table1: Selected soil characteristics

	pH_{CaCl2}	N total	C org	$CaCO_3$	sand	silt	clay	Zn_{HNO_3}	Zn_{NaNO_3}
		g kg^{-1}						mg kg^{-1}	
Le Locle	7.9	3.1	111	413	342	452	207	890	nd*
Dornach	7.6	10.3	44	137	182	500	318	630	0.081
Giornico	4.5	4.3	28	0	725	206	69	273	16.33

*nd: not detected

2. Results and Discussion



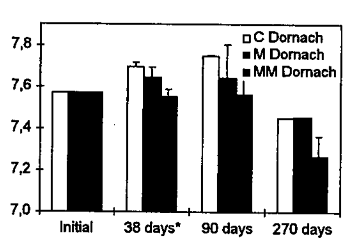
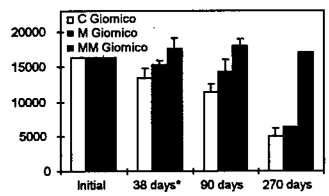


Figure 1: pH in Giornico and Dornach soils planted with *Salix aurita* with or without NH_4Cl addition. C=control, M=0.01M NH_4Cl, MM=0.1M NH_4Cl. *: NH_4Cl was applied 10 days before the 38 days-measurements.

pH: A decrease in pH is observed in the treated pots 10 days after NH_4Cl addition for Dornach and Giornico soils. The effect is still visible after 270 days and pH is always lower for the 0.1M NH_4Cl treatment (figure 1). A pH decrease over time is also observed for the 0.1M NH_4Cl treatment. In that case, the plants died and

the decrease is due to the sole effect of NH_4Cl on the soil (ion exchange and NH_4 nitrification). There is no change in pH in Le Locle soil.

$NaNO_3$ extraction: NH_4Cl has also an effect on Zn_{NaNO_3} which is larger in the treated pots than in the control (figure 2), probably due to a pH decrease, an increase in the readily soluble Zn-chloride species as well as a desorption of Zn from exchangeable sites. However, whereas the Zn_{NaNO_3} stock in Giornico decreases over time (apart from the highest treatment for which the plants died), in Dornach the effect is not visible probably due to lower concentrations. In Le Locle Zn_{NaNO_3} is not detected whatever the treatment.



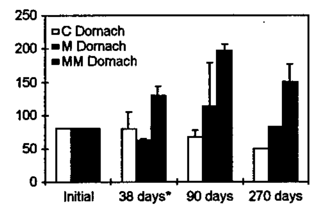
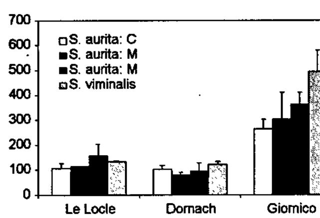
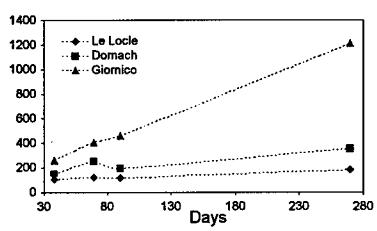


Figure 2: Zn extracted by 0.1M $NaNO_3$ in Giornico and Dornach soils planted with *Salix aurita* with or without NH_4Cl addition. C=control, M=0.01M NH_4Cl, MM=0.1M NH_4Cl. *: NH_4Cl was applied 10 days before the 38 days-measurements.

Plant uptake: The increase in plant uptake due to the additive is measureable for willows grown in Le Locle and Giornico soils 10 days after NH_4Cl addition, is still observed after 90 days (data not shown), but is hidden after 270 days by interplant variations and a general increase in Zn uptake efficiency. The latter is due to an increase both in biomass and Zn concentrations in all plants in all 3 soils (figure 4). However, concentrations in *Salix aurita* are always lower than a reference, *Salix viminalis*. Zinc concentrations are maximal in leaves of *S. aurita* grown in Giornico soil (up to 2400 mg kg^{-1} Zn in DM as compared to 900 mg kg^{-1} Zn in DM in Dornach). The lowest concentrations are found in roots. The Zn repartition between leaves, stems and roots doesn't change whatever the treatments, the soil type or sampling time. Thus it is related neither to the soil characteristics nor to Zn status in the soil. No relation is found after 270 days between the uptake and the available pool ($NaNO_3$) in the soil.

Figure 3: Zn concentrations in shoots of *Salix aurita* after 10 days with and without NH_4Cl addition





and comparison with *Salix viminalis*.

Figure 4: *Salix aurita* uptake kinetics: Zn concentrations in shoots without NH_4Cl addition and over 270 days.

4. Conclusions

Ammonium chloride applied to three different soils increases Zn availability, as shown by the $NaNO_3$ extraction and the plant uptake. But after 270 days, although more Zn is still available for plants in the treated pots, part of the mobilised Zn is reincorporated into the soil: indeed, plants account for only ca. half of the decrease in Zn_{NaNO_3} in Giornico and Dornach soils. On another hand, in Le Locle soil, *Salix aurita* does accumulate Zn although there is no significant Zn_{NaNO_3}. Thus, Zn extracted by $NaNO_3$ is not correlated with the plant uptake. Also, *S. aurita* accumulates Zn in its shoots and its uptake efficiency increases with time, hidding partially the effect of NH_4Cl. In conclusion, both NH_4Cl doses have a positive short term effect on plant uptake and a positive long term effect on pH and available Zn in the soil, but the response is stronger in the acidic soil with high Zn_{NaNO_3} concentrations (Giornico). In Le Locle soil, large organic matter and carbonate contents might be responsible for a large soil buffer capacity.

EFFECT OF NUTRIENT SOLUTION COMPOSITION AND BUFFERED CD^{2+} ON YIELD AND CD LEVELS IN RICE GRAIN

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1. Introduction

Cadmium has caused chronic toxicity to humans. The safe levels of Cd in cereal grains and other food products are still disputed and further animal feeding studies are necessary to explore rice grain Cd bioavailability. The purpose of this study was a) to develop a method to obtain rice grain with intrinsic Cd for animal feeding studies and b) to study Cd uptake and distribution within rice plants grown in nutrient solution. Grain with labeled Cd at 1 mg kg^{-1} will be produced for feeding studies.

2. Materials and Methods

Rice plants cv. Jefferson were grown to full maturity in two different nutrient solutions used by others to produce rice grain: M. Grusak - solution A, and M. Chino - solution B. Solution A: Ca 0.5, Mg 0.5, K 0.5, S 1.0, Cl 1.0, NH_4 1.0, NO_3 2.0, P 0.5, Si 0.1 mM; and Mn, Cu, Zn, Ni, Fe, B and Mo 1.0, 0.5, 2.0, 0.1, 10.0, 20.0 and 0.1 μM, respectively. Solution B: Ca 0.36, Mg 0.46, K 0.54, Na 0.34, S 0.73, Cl 0.72, NH_4 0.32, NO_3 0.32, P 0.18, Si 0.17 mM; and Mn, Cu, Zn, Fe, B and Mo 4.61, 1.56, 1.53, 20.0, 18.4, and 0.1 μM, respectively. Solutions were changed every 6 days. Cd treatments were applied at anthesis at 66 days of growth; Cu concentration in both solutions was also increased to 2.0 μM with the buffering. At the time of Cd treatment initiation, plants grown in solution B reached the stage of panicle formation while plants grown in solution A reached this stage 6 days later. Cd was applied at concentrations of 0, 0.5 and 2.0 μM; Cd activity was buffered by 50 μM excess of EGTA. Free Cd activity (pCd^{2+}) computed for 0.5 and 2.0 μM Cd treatments were 11.16 and 10.56 for solution A, and 11.09 and 10.49 for solution B. The pH of growth solution was recorded daily and adjusted to pH 6.0, if necessary, by addition of 0.1M NaOH. Plants grown in solution B reached full maturity earlier and were harvested after 108 days of growth in nutrient solutions followed by plants grown in solution A which were harvested after 116 days of growth. At harvest, the length of the longest shoots and roots of each plant were measured, numbers of tillers recorded, roots were washed and plants were separated into roots, shoots, ear leaf blades, remaining leaf blades and heads. Heads were further separated into brown rice and hulls. Content of Cd, micro- and macronutrients in plant samples was determined by flame AAS and ICP after dry ashing.

3. Results and Discussion

The composition of the growth medium had a great impact on plant growth. Solution A produced plants with a greater number of tillers, shoot biomass and grain yield while the root biomass was similar for both solutions (Table 1). Plant growth and grain yield were not affected by Cd treatments. A linear relationship was obtained between Cd concentrations in the solutions and Cd concentrations in brown rice (Figure 1). Solution A resulted in much higher Cd concentration in grain in comparison to solution B at the corresponding treatments. The same tendency was expressed in all analyzed plant parts. Differences in Cd uptake by plants grown in different growth media cannot be explained by the small differences in Cd^{2+} activities (at corresponding Cd treatments) but may be rather attributed to the

differences in composition between solutions A and B. At the higher Cd treatment, plants grown in the solution A produced grain with Cd level close to the desired 1 mg kg^{-1}.

Table 1. Rice response to nutrient solutions.

Growth Solution	Number of Tillers/plant	Highest Tiller Length	LONGEST Root Length	Shoot dry weight	Root dry weight	Grain yield dry weight
		------------	cm ------------	------------	g / 4 plants	------------
A	20	69.4	47.4	214	18.8	119
B	12	59.9	71.2	97	20.8	77

The linearity of the relationship between Cd in solution and Cd in grain allows for an easy adjustment of Cd in solution to produce the desired level of Cd in brown rice.

The content of Zn, Cu, Fe, Mg, Ca and K in brown rice was similar for both solutions but Mn was higher in B grain. Zn level in A and B brown rice was double the maximum reported for US brown rice (Wolnik et al., 1983). Mn concentrations in brown rice A and B were similar to the average Mn levels. The levels of all other macro- and micronutrients analyzed corresponded with the highest values found in commercially grown American brown rice.

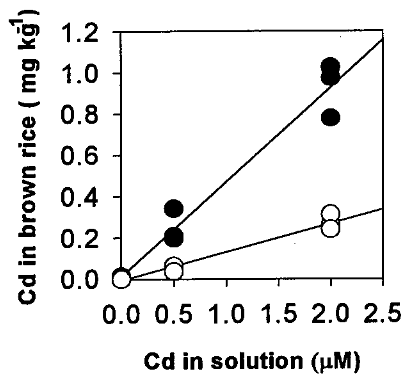

Figure 1. The effect of Cd in solution on the concentration of Cd in brown rice grown in two nutrient solutions (•, solution A; o, solution B).

4. Conclusions

The chelator-buffered solution culture allowed us to obtain rice grain with the desired Cd concentration. However, the brown rice was higher in several elements than US commercial brown rice.

5. References

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THE EFFECTS OF SOIL TREATMENT AND PLANT SPECIES ON ROOT GROWTH AND METAL UPTAKE IN ZINC-CONTAMINATED SOILS

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1. Introduction

It is hard to revegetate Zn-contaminated hillside lands because the steep slopes make it difficult or impossible to use agronomic equipment to incorporate amendments for ameliorating the toxic soil metals. Two previous studies have shown that surface applications of biosolids were successful in promoting plant growth for up to two years on hillside land contaminated by air emissions originating from a metal smelter (Sopper, 1989). We hypothesized that depth and intensity of rooting are major factors in the long-term survival of plants grown on contaminated soils covered with a clean soil or organic material. However, compared with top growth, little is known about Zn uptake and the rooting characteristics of plants grown on high-Zn soils or the combined effects of plant species and high-Zn soil on root and shoot development. The objective of this research was to evaluate shoot morphology, rooting ability, and metal uptake of four cool-season grasses grown on a high-Zn soil covered with either a fine-textured topsoil or a mixture of biosolids, fly ash, and dolomitic limestone.

2. Materials and Methods

To study plant growth on Zn-contaminated soils, we used a greenhouse experiment with a completely randomized split-plot experimental design, containing four replications each of four grasses and three soil treatments. The mean DTPA extractable metals in the soils were 1325 μg/g Zn, 35 μg/g Cd, and 49 μg/g Pb. One soil treatment contained a 45-cm section of Zn-contaminated soil covered with a 5-cm layer of a mixture of biosolids, dolomitic limestone, and fly ash (BP). Another treatment consisted of a 5-cm layer of the biosolids mixture, above a 22.5-cm layer of uncontaminated topsoil, above a 22.5-cm layer of Zn-contaminated soil (BCP). The third treatment consisted of a 50-cm layer of uncontaminated topsoil (C). The soil at the smelter site was previously described by Sopper (1989). The BP treatment is similar to that being used to revegetate the smelter site. A slant-tube technique was used for evaluating shoot and root growth of four dominant species sown and existing at the smelter site; the tubes and the plant data collection and analysis procedures were previously described by Palazzo and Brar (1996). The grasses studied were Reliant hard fescue (*Festuca ovina* L.), Oahe intermediate wheatgrass [*Agropyrun intermedium* (Host) Beauv.], Ruebens Canada bluegrass (*Poa compressa* L.), and Tribute tall fescue (*Festuca arundinaceae* Schreb.). These grasses represent the.

3. Results and Discussion

<u>Shoot-Zn Concentrations</u>. Plants grown in the BP soil treatment had greater Zn concentrations than the plants grown in either the C or BCP treatments. The species with the greatest concentrations of Zn were tall fescue (1,553 μg/g), Canada bluegrass (1,166 μg/g), and wheatgrass (1,130 μg/g); hard fescue had the lowest (422 μg/g). With the BP treatment, all grasses except hard fescue contained Zn levels above 500 μg/g, which is considered toxic to plants (Chaney, 1993). Uptake of Zn in the

BCP treatment was about 25% of the BP treatment. The greater depth to the Zn-contaminated layer in the BCP treatment compared to the BP treatment apparently reduced the Zn uptake by plants. No visual symptoms of plant Zn phytotoxicity was observed in any of the species.

Root Growth. The two soil treatments that included biosolids as a soil amendment produced the most roots compared to the clean-soil control. Most of the roots were found in the 0- to 5-cm biosolids layer in both treatments due to the additional nutrients and water holding capacity of the biosolids. Wheatgrass and tall fescue produced a greater quantity of roots than either hard fescue or Canada bluegrass in the biosolids treatments. Therefore we define the first two species as being stronger rooting plants than the latter two species. Palazzo and Brar (1996) have also shown that tall fescues produce a greater root mass than hard fescue in non-contaminated or clean soils.

In the Zn-contaminated layers of the BP and BCP treatments, wheatgrass had the greatest root area and weight densities. Root growth was about three times greater than tall or hard fescue. Hard fescue had low root weight production in all layers, but showed only a minor reduction in growth in the contaminated soil. Root samples taken from the field site confirmed that wheatgrass was better able to root into the contaminated soil than Canada bluegrass.

4.Conclusions

Wheatgrass produced deeper rooting into the contaminated layer which should help this species persist on Zn-contaminated sites when the biosolid layer is decomposed. The higher shoot-Zn concentrations in the BP treatment compared to the BCP treatment were directly related to the much shallower depth (5 cm) of the contaminated soil in the BP treatment. Hard fescue had the lowest Zn concentrations in shoot tissue and could be termed a Zn excluder. Thus, hard fescue and wheatgrass should be the best long-term survivors in high-Zn soils, with hard fescue effectively excluding toxic Zn uptake. Also, improper selections will be difficult to identify since phytotoxic symptoms were not present.

5. References

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EFFECT OF SOIL ACIDIFICATION BY FERTILIZER APPLICATION ON HEAVY METAL MOBILITY:UPTAKE BY WILLOW AND LEACHING

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1. Introduction

. The **objective** of this study is to increase the mobility of heavy metals selectively in the rhizosphere in order to increase plant uptake of heavy metals and to prevent leaching from the bulk soil. We tested the following hypotheses: 1) Mobility and plant uptake of heavy metals will be increased by ammonium fertilizers; 2) Placement of ammonium fertilizers will minimize losses of heavy metals by leaching and increase uptake by lowering pH locally; 3) Adding nitrification inhibitors will prevent acidification of and heavy metal leaching from the bulk soil.

2. Materials and Methods

A willow clone (Salix viminalis *'Jorr'*) which is known to take up comparatively high amounts of heavy metals was planted in pots, filled with 4.3 kg of soil (2 mm sieved) and grown in a glasshouse over 17 weeks in 5 replicates. The soils were kept humid and once a heavy rainfall was simulated (118 liters m^2) to cause leaching of heavy metals Two different soils contaminated with heavy metals at compara-ble total amounts (Fliseryd, industrially contaminated; and Klärschlamm, 'KS', long term sewage sludge application) were tested (Table 1). Mobile Zn contents were in the same range but Fliseryd contained much more mobile Cd.

Table 1:Properties and heavy metal contents of the test soils

soils	C_{org}	N	lime	pH	CEC eff	Cd total *	Cd mobile §	Zn total *	Zn mobile §
	%	%	%		mM kg^{-1}	mg kg^{-1}	µg kg^{-1}	mg kg^{-1}	µg kg^{-1}
Fliseryd	2.3	0.2	0.0	4.8	62.7	2.4	256	104.8	988
KS	1.8	0.2	0.5	6.4	163.7	1.9	10	288.1	807

*: digested with aqua regia (ISO 11466.2) using microwave; §: 1 part soil extracted with 2.5 parts of 1 M ammonium nitrate over 2 h (DIN 19730), centrifuged by 1500 rpm

The different treatments were a) mixing ammonium sulfate with the soil material; b) like a), but with additional nitrification inhibitor (DCD); c) ammonium sulfate fertilizer plus DCD which was placed in 15 cm depth (CULTAN; *Sommer, 1990*); d) ammonium nitrate; all treatments were calculated to give 100 mg kg^{-1} fertilizer-N. After 10 weeks total Zn and Cd contents in old leaves were measured. Plants were harvested 17 weeks after planting.Mobile Zn and Cd were measured in rhizosphere soil (approx. 2 mm diameter around the root) and in bulk soil, and of Cd leached out from the pots was measuredby graphite furnace and flame AAS (SpectrAA series 800 and 200, Varian). Analysis of variance was performed with SAS programme (SAS Inst., 1985); differences between means were calculated by Tukey's studentizied range test with α=0.05.

3. Results and Discussion

Plant growth was not significantly different between treatments, but older leaves of CULTAN-fertilized plants became necrotic. Zn content of old leaves of the plants grown on KS soil was increased by ammonium sulfate fertilization (table 2), but also with ammonium nitrate fertilizer Zn contents were in toxic range (*Kabata-Pendias and Pendias,* 1992). On Fliseryd soil ammonium sulfate did not increase mobile heavy metal contents significantly; here Cd contents of old leaves generally were in toxic range. In Fliseryd soil mobile heavy metal contents were selectively increased in the rhizosphere soil when ammonium sulfate was

applied, but leaf heavy metal contents were only slightly elevated when ammonium sulfate was mixed with soil without DCD. In KS mobile heavy metal contents in rhizosphere were significantly increased after CULTAN fertilization; mobile Cd contents were increased also after ammonium sulfate fertilization, compared to ammonium nitrate treatment, respectively.

Table 2:Contents of Zn and Cd in soil, old leaves and amount leached after irrigation

treatment	mobile heavy metals in soil Cd		Zn		total heavy metals in old leaves		Cd leached after irrigation
	rhizo-sphere	bulk soil	rhizo-sphere	bulk soil	Cd	Zn	
	mg kg^{-1}				mg kg^{-1}		µg per pot
			Fliseryd soil				
ammonium sulfate	0.37^{a}	0.21^{a}	1.40^{a}	0.84^{a}	11.22	85.7	7.32^{a}
ammonium sulfate + DCD	0.30^{b}	0.24^{a}	1.20^{a}	0.91^{a}	4.55	66.1	1.47^{bc}
Cultan	0.32^{ab}	0.23^{a}	1.29^{a}	0.92^{a}	8.19	78.3	4.20^{b}
control	0.24^{c}	0.23^{a}	0.70^{b}	0.91^{a}	8.42	73.8	0.32^{c}
			KS soil				
ammonium sulfate	0.023^{a}	0.018^{a}	2.28^{ab}	1.95^{ab}	1.83	364	0.21^{d}
ammonium sulfate + DCD	0.017^{ab}	0.014^{a}	2.15^{ab}	2.03^{ab}	1.16	462	0.18^{d}
CULTAN	0.025^{a}	0.019^{a}	2.74^{a}	2.39^{a}	1.86	457	0.21^{d}
control	0.010^{b}	0.019^{a}	1.11^{b}	0.95^{b}	0.92	239	0.15^{d}

In KS soil mobilization of Zn by CULTAN treatment was more pronounced than in Fliseryd soil (2.5 compared to 1.8). In contrast to Fliseryd in the bulk soil of KS mobilization of Zn was enhanced by acidifying fertilizer application and solely after ammonium sulfate treatment rhizosphere Cd contents were significantly higher than in the bulk soil.
After a simulated heavy rainfall significantly more Cd was leached from Fliseryd than from KS soil. Twice the amount of Cd was leached from Fliseryd soil when non-acidifying fertilizer was given compared to KS soil, but after ammonium sulfate fertilization up to 35 fold more Cd was leached.
On acid Fliseryd soil acidification did not lead to increased heavy metal contents in the plant material but to mobilization in the rhizosphere and to tremendous Cd leaching. On KS soil the heavy metal contents in the plant material could be increased by soil acidification and mobile heavy metal contents in the soil were increased, but far less Cd was leached compared to Fliseryd soil and there was no effect of acidification on leaching. Adding nitrification inhibitors to ammonium sulfate fertilizer reduced Cd contents in plant material and Cd leaching from the acid Fliseryd soil.

4. Conclusions

Phytoremediation strategies others than acidification should be tested to reduce heavy metal contents in Fliseryd soil. Further analyses of plant material will reveal the suitability of acidification methods as phytoremediation strategies on KS soil.

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WHERE DO PLANTS TAKE UP THEIR NICKEL FROM?

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1. Introduction

Until recently, the use of isotopic exchange methods in soil plant studies was mostly limited to macronutrients (e.g. P or N) and sometimes to micronutrients such as Zn. Among these methods, isotopic exchange kinetics (IEK) allowed to assess both phytoavailability of unessential trace elements in soils and dynamics of their supply to plants over time (ECHEVARRIA *et al.*, 1998). Equivalence between the isotopically-exchangeable and the phytoavailable trace element was shown for Ni (ECHEVARRIA et al., 1998; SHALLARI, 1997), for Zn (SINAJ et al., 1998) and for Cd (GÉRARD et al., 1999).

This work was designed to show the power of IEK in the study of Ni phytoavailability: description of the pools of isotopically exchangeable Ni as a function of time of exchange, study of the plant behavior and its effect on the phytoavailability of Ni after culture, and validation of chemical extraction techniques, which are more adapted to routine conditions.

2. Materials and Methods

Red clover (*Trifolium pratense* L.) and *Alyssum murale* (L.), a Ni hyperaccumulator, were grown on three soils, two agricultural soils - S_V and S_N -(respectively 24 and 33 mg Ni kg^{-1}) and one serpentine industrial soil (1316 mg Ni kg^{-1}) -S_P. Plants were harvested after three months. Samples of the three soils were taken just before sowing and after harvest (supposing that pot soil was mostly rhizosphere soil) and were air-dried before performing IEK (protocol in ECHEVARRIA et al., 1998). From IEK, representations of phytoavailable Ni were made using the multi-compartment model (FARDEAU, 1993).

A. murale and clover were also grown on the same three soils that where spiked with ^{63}Ni to compare the absorption behavior of the two plants and to verify whether hyperaccumulating plants take up Ni in the isotopically exchangeable pool such as non-accumulating species, or in other non-exchangeable pools by some unknown solubilization methods. Extraction of soil Ni after harvest were performed with two chemical reagents, NH_4NO_3 1M and DTPA. The isotopic compositions (IC_P i.e. ^{63}Ni/total Ni) of Ni in plants, of Ni extracted by the two reagents(IC_E), and Ni in the soil solution from IEK experiments (IC_P) were determined.

3. Results

Ni was mainly under unexchangeable forms in the three soils, except for S_V, where almost 20% of total Ni was exchangeable up to harvest time. On this soil, *A. murale* took up more than 17% of this phytoavailable Ni. The schemes representing phytoavailability of Ni in the serpentine soil show that after culture red clover had dramatically increased phytoavailability of Ni whereas *A. murale* had little influence on this parameter (Figure 1).The effect of clover was mainly an increase of the more rapidly exchangeable pools through the increase of the concentration of Ni in the soil solution (C_{Ni}). However, no significant changes in pH could be detected, although there was a trend in rhizosphere acidification under the presence of clover. The ability of clover to increase Ni phytoavailability in

such a Ni-rich soil has to be considered with great attention for the management of such soils. IEK thus allowed to assess the effect of two plants, however, mechanisms remain to be elucidated.

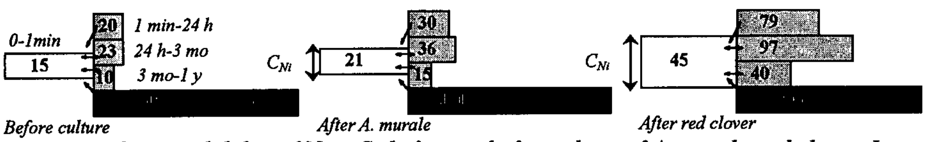

Figure 1: Phytoavailability of Ni in S_P before and after culture of A. murale and clover. Isotopically exchangeable Ni is represented for 5 time intervals (mg Ni kg^{-1} soil)

Comparison of the IC_P of Ni for the three soils showed that clover and *A. murale* took up Ni in the same pool which was the isotopically exchangeable pool. Therefore, *A. murale* did not display special mechanisms to mobilize unavailable Ni as it was sometimes suspected but depleted soil isotopically exchangeable Ni. Studies carried out with a hyperaccumulator of Zn and Cd, *Thlaspi caerulescens*, also showed that this plant takes up Cd from the isotopically exchangeable pool (GÉRARD et al., 1999).

$IC_{E\ (DTPA)}$ were similar to IC_P on the three soils but $IC_{E\ (NH4NO3)}$ showed greater discrepancies, indicating that DTPA could be a useful reagent for the extraction of phytoavailable Ni in soils (ECHEVARRIA et al., 1998; SINAJ et al., 1998).

4. Conclusions

The different phytoavailable pools of Ni in three soils as a function of their time of exchange were compared before and after the uptake of Ni by plants. This made it possible to assess the effect of plant culture on the further phytoavailability of Ni. The behavior of *A. murale* was not different from that of clover, and IEK showed that this species did not solubilize unavailable forms of Ni in the soil. The high uptake efficiency of *A. murale* was maybe due to a better capacity to take up Ni at low concentrations in the soil solution. DTPA was shown to be a good model to assess truly phytoavailable Ni in soils, but further studies should try to correlate the amount extracted with given exchangeable pools. The development of simple chemical extractions in agreement with true bioavailability is a major application for IEK.

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LIMING REDUCES CADMIUM UPTAKE FROM SOIL: WHY DOESN'T IT WORK BETTER?

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1.Introduction

Liming is a widely recommended strategy to reduce mobility and plant availability of soil contaminants such as cadmium (Cd). Field data often show limited success of liming on reducing Cd levels in crops (Andersson and Siman, 1991). The lack of a consistent negative relationship between soil pH and Cd availability contrasts the generally negative relationship between soil pH and Cd mobility. Most Cd sorption models predict a 4-6 fold decrease in metal activities in soil solution per unit pH increase. In this work, liming effects on Cd levels in soybean plants are compared with metal activities in soil solution. In addition, pH effects on root uptake of Cd are measured in solution culture.

2.Materials and Methods

Pot trial. The toplayer of a silt-loam soil (74% silt, 5% clay, 2.9 % organic matter, 0.4 mg/kg total Cd, 68 mg/kg total Zn) was sampled in northern Wisconsin (U.S.), sieved (2 mm) and homogeneously limed with CaO at three rates varying from 0.5 to 2.8 g/kg. All soils were fertilised with KNO_3 (1.8 mmol/kg) and KH_2PO_4 (1 mmol/kg). After one month equilibration (30 % water content) soils were re-mixed and potted (2.3 kg/pot; three replicates per treatment). Soybean (*Glycine max* cv. Corsoy) was grown in a greenhouse for 3 weeks. The shoot Cd and Zn content was measured by ICP-MS (HNO_3 plant digests). After harvest, soils were wetted to 42 %, equilibrated overnight and soil solution (saturated paste extract) separated by direct centrifugation (1300 g, 45 min). The solutions were membrane filtered (0.22 µm). Free metal ion concentrations were measured by Donnan dialysis of the soil solution (Helmke et al., 1997). Metal concentrations (^{111}Cd and ^{66}Zn) in the dialysed fraction were determined by ICP-MS.

Solution culture Soybean seedlings were grown for 11 days in nutrient solution in a greenhouse. Solution pH was varied at three levels between 5.1-7.1with two replicates. Solutions contained either no chelating agent or contained 25 m*M* Nitrilo-Triacetic Acid (NTA). Solution Cd was maintained at 25 n*M* and the Cd free metal concentration (Cd^{2+}) was either unaffected by the pH (-NTA) or decreased 5.3 fold between pH 5.1 and pH 7.1 (+NTA).

3.Results and Discussion

Liming the soil increased soil pH ($CaCl_2$) by two units. The shoot Cd and Zn concentrations both decreased less than 3-fold by liming (Fig.1). No significant treatment effect was found on plant growth (not shown). Free metal ion concentrations in soil solutions, however, decreased 10 fold (Cd^{2+}) and 30 fold (Zn^{2+}). Preliminary solution culture work showed that plant Cd content is proportional to solution Cd^{2+} up to $10^{-7}M$. It can be hypothesised that the lack of a more pronounced

decrease in Cd uptake upon liming must be related to varying ionic interactions in Cd^{2+} uptake from soil solution.

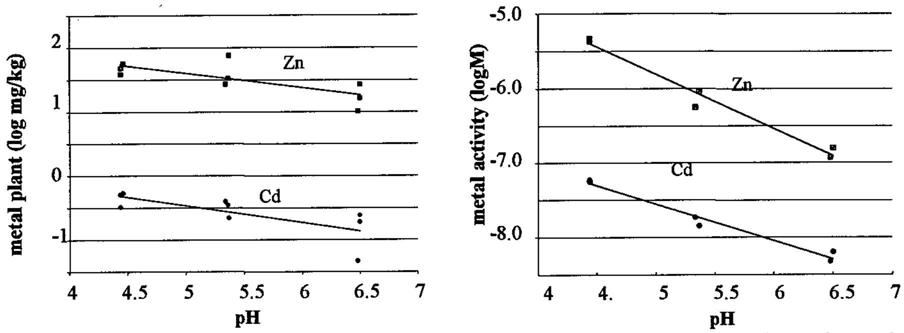

Fig. 1. Semi-log plots of the effect of soil pH on Cd and Zn concentrations in soybean shoots (left) or on measured free metal ion concentrations in soil solution. Results of a pot trial experiment in which soil was limed at two rates + control. Note the lower relative effect (i.e. lower slope in semi-log plot) of the pH on shoot metal concentrations than on free metal ion concentrations.

Results of solution culture (Fig. 2) studies support this hypothesis. Increasing solution pH *increases* shoot Cd content both in solutions with constant solution Cd^{2+} concentration (-NTA) or in solutions with 5-fold decreasing Cd^{2+} concentrations (+NTA). The difference in the response of Cd uptake to varying pH between the -NTA and +NTA solutions cannot simply be predicted based on the difference in Cd^{2+} activities in both solutions. It is now hypothesised that pH increases the Cd^{2+} uptake capacity of a root due to altering H^+ and Zn^{2+} concentrations in solution.

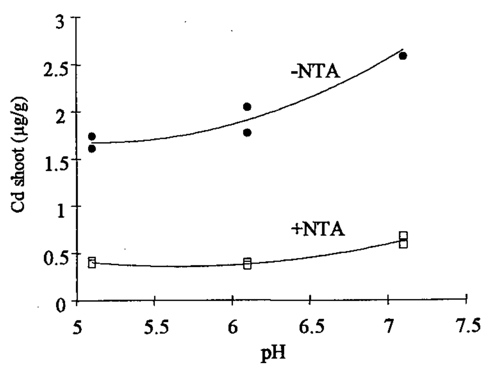

Fig.2 Solution culture data: cadmium in soybean shoots at varying pH in the presence or absence of the chelator NTA in solution

4. Conclusions

Liming a soil has a more pronounced effect on Cd^{2+} and Zn^{2+} activities in soil solution than on levels of Cd and Zn in plants grown on that soil. Solution culture data shows that root uptake per unit Cd^{2+} and Zn^{2+} in solution increases with increasing solution pH.

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CONTAMINATION AND BIOAVAILABILITY OF HEAVY METALS ALONG URBAN-RURAL LAND-USE IN CENTRAL TAIWAN

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1. Introduction

Urban-rural land-use gradients are one of the environmental factors influencing the soil quality. Although gradients of land use can be measured, little is known about the effect of urbanization on urban-rural soil contamination. Therefore, an urgent understanding of the contamination between urban and rural soils has arisen so as to can better develop an assessment technique to integrate the national land-use policy.

A simplest way to assess the soil contamination is to compare the contents of metals with the promulgated standard values. Also, bioavailability of heavy metals can be use as the ecotoxicity guideline due to that the microbial activity would be significantly reduced when soils were severely contaminated with heavy metals. In this study, the contents of heavy metals along urban-rural areas in combination with the microbial density were analyzed to establish the relationship between metal and microbial concentrations. Moreover, the bioavailabilities of metals and the sensitive metals to soil contamination were elavuated.

2. Materials and Methods

Two sampling areas, Da-lee City and Da-ya Village, adjacent to Taichung metropolitan in central Taiwan were selected to represent the urban and rural areas, respectively. 15 samples in Da-lee City and 40 samples in Da-ya Village were collected. All samples were air-dried and sieved with 20-mesh sieve. The 0.1N HCl extractable concentration of heavy metals were performed by containing 4g soil with 40 ml 0.1 HCl solution in a 50-ml centrifuge tube. Microwave digestion was used to determine the total amounts of heavy metals in soils. All of the supernatants were filtrated with 0.45μm filter and analyzed by ICP-MS for Ni, Zn, Cd, Cr, Pb, As and Cu. For bioavailability experiments, plate count and AODC epifluorescence technique were used to determine the activity of microbes in soils.

3. Results and Discussion

Table 1 illustrates the concentration ranges and mean levels of heavy metals extracted by 0.1N HCl in urban and rural areas. Analytical results showed that urban area was highly polluted with heavy metals. The average concentrations of Ni, Zn, Cd, and Cr were 28.05 mg/kg, 37.57 mg/kg, 0.97 mg/kg, and 11.65 mg/kg, respectively. These values are sufficient high to trigger an emergency cleanup. Also, 20% to 100% of the tested samples, depending on the type of heavy metals, exceeded the standard values promulgated by Taiwan EPA. For rural area, the concentrations of Ni, Cu, Zn, Cd and Cr were lower than those in urban area. The high detection levels of As in rural area may be due to the usage of agricultural products. Nickel and cadmium were the often found metals that the detected levels exceeded the standards. The frequencies exceeding the standards of Ni and Cd were 57.5% and 100 %, respectively.

No significant difference in microbial density between urban and rural areas was observed. The observed cell numbers in urban area ranged from 4.95×10^7 cells/ml to 18.25×10^7 cells/ml, whereas cell densities of 1.2×10^7 cells/ml to 19.25×10^7 cells/ml were obtained in rural area. As illustrated in Figure 1, a contour relationship of the spatial distribution of microbial density in urban area was illustrated. Similar distribution pattern also obtained in rural area, showing that microbes can undergo biosorption processes and increase the bioavailabilities of heavy metals. The relationship between heavy metal and microbial density along urban-rural soil shown in Figure 2 demonstrates that nickel, zinc and cadmium were the sensitive metals to the bioavailability and to soil contamination.

4. Conclusions

Results obtained in this study depict that urbanization is a major environmental factor for soil contamination. The high contents of heavy metals in urban area were sufficient to trigger an emergency cleanup. Also, the relationship between metals and microbial density demonstrated that nickel, zinc and cadmium were the most effective metals on urban-rural soil contamination.

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Table 1. The concentration ranges and means levels of heavy metals in urban and rural soils

Item	Concentration ranges (mean level) of heavy metals (mg/kg)						
	Ni	Cu	Zn	Cd	Pb	As	Cr •
Standards#	12	26	25	0.43	18	—	12
Da-li City	2.45-112	2.45-97.4	14.8-110	0.61-1.64	8.73-41.9	0.15-0.55	1.64-68.1
	(28.05)	(21.67)	(37.57)	(0.97)	(15.33)	(0.25)	(11.65)
Da-ya Village	2.44-54.6	0.92-64.9	11-73.5	0.57-1.07	11.7-22.1	0.13-1.03	1.34-18.6
	(16.21)	(15.86)	(11.75)	(0.82)	(15.96)	(0.46)	(6.11)

#: data from Chen et al. (1996).

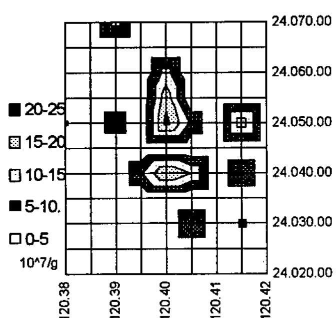

Figure 1. The distribution of microbial density in urban area(Da-lee City).

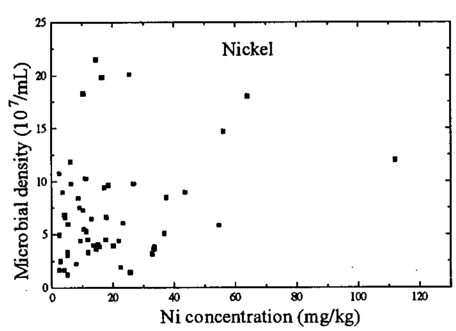

Figure 2. Relationship between microbial density and nickel concentration along urban-rural soils.

INCREASED ZINC-EXTRACTION POTENTIAL OF MAIZE BY ENHANCING METAL AVAILABILITY IN SOIL

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1. Introduction

Phytoremediation is a technique which uses the ability of plants to extract heavy metals from soil. To remove sufficient amounts of heavy metals by this technique, both high metal concentrations in the plant and high biomass yields are important. The largest part of heavy metals in arable soils is adsorbed on soil particles and is not available for plants. Therefore a controlled increase of the plant available heavy metal fraction in soil is proposed. The plant available heavy metals in soil can be increased by decreasing soil pH or by augmenting chelating agents. By using plants to extract metals from soil, the concentration of mobile, and therefore plant available metals must be adapted to the uptake capacity of the particular plant. In a first step the potential phytoextraction of zinc from soils using high yield varieties, such as maize and tobacco, has been investigated. In a second step the increase of plant available metals in soil will be attempted using organic mobilising agents, such as NTA (Nitrilotriacetic acid) and Citric acid.

2. Methods and Materials

Eighteen field plots of 7.8 m2 were set up in 1987. The plots were treated with graded amounts, 0, 50, 150, 350, 750 and 1550 mg/kg of zinc-sulphate ($ZnSO_4.7H_2O$), each on three replicates. From 1988 to 1995 the effect of increasing zinc content on the yield and quality of fodder plants was investigated. Uniform doses of N-fertiliser (NH_4NO_3) were applied to each plot. From 1987 to 1997 the average total HNO_3-extractable zinc content in surface soil of all treated replicates decreased to maximum concentrations of about 400 mg/kg (dry matter soil), probably as a result of zinc transfer to deeper soil layers. On the contrary the mobile ($NaNO_3$-extractable) zinc content of the topsoil considerably increased. The average $NaNO_3$-extractable zinc contents in soil (from three replicates) are shown in Table 1. The increase of mobile zinc is presumably caused by the pH decrease from around 6 to 5 from 1988 until 1996. In 1996 the plots have been used to investigate the ability of maize (Zea mays, cv. LG 11) to remove zinc from soil. In 1997 on half of each field plot maize and tobacco (Nicotiana tabacum, cv. Badischer Geudertheimer) were planted. During the vegetative period soil and plant samples were taken from all plots at different growth stage. Soil samples were collected from the topsoil (0-20 cm depth) in the root area of the collected plants and analysed for mobile zinc and pH. Plant samples were taken randomised, three from each plot, and analysed for zinc content as well.

3. Results and Discussion

Zinc concentration in maize shoots almost linearly increased with increasing mobile zinc content in soil (Figure 1). In contrast the zinc concentration in the plant tissues of the first sampling (after 55 days) markedly decreased in the second (after 82 days) and in the third (maturity of maize), probably as a result of growth dilution (Fig. 1). However zinc removal is at its highest after 82 days of growth period, when maize has already started to develop its fruit and is in the growth stage of early milk (Table 1). For remediation purpose maize should be harvested before full grain ripeness. The total zinc removal by maize at the 'early milk' growth stage (Table 1) corresponds to the average removal of zinc (4.6-6.4 kg ha^{-1}) reported by MC GRATH (1998) using the hyperaccumulator Cardaminopsis halleri.

4. Conclusions

Increasing the available zinc content in soil led to a considerable increase of zinc concentration in maize shoots. But during growth period the zinc content in maize strongly decreases. Results of one year experiments showed that the maximum removal of zinc, using the high yield variety maize, could be achieved after 82 days of growth period. Further experiments will provide more information on the optimum time for yield..

0Presently, experiments are in progress to investigate the possibility of adding organic mobilising agents to soil to increase the mobile metal content.

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Table 1: Concentrations of Zn (mg kg^{-1} dry matter), yields (t ha^{-1}) and removals (kg ha^{-1}) during growth period of maize by varying mobile ($NaNO_3$-extractable) zinc contents in soil (average and standard deviation of three replicates)

NaNO3-extractable zinc in soil (mg/kg dm)	Maize 55 days growth period			Maize 82 days growth period		
	Conc.	Yield	Removal	Conc.	Yield	Removal
1.7 (± 0.1)	69.6 (± 7.9)	4.8 (± 0.4)	0.3	49.2 (± 1.5)	15.8 (± 1.9)	0.8
5.6 (± 1.0)	189.2 (± 28.5)	4.8 (± 1.0)	0.9	118.2 (± 18.9)	15.2 (± 1.4)	1.8
13.2 (± 2.7)	326.7 (± 50.5)	3.9 (± 1.3)	1.3	234.7 (± 32.0)	16.7 (± 1.1)	3.9
25.6 (± 5.1)	625.1 (± 96.1)	4.4 (± 1.0)	2.8	391.7 (± 34.1)	17.2 (± 3.0)	6.7
44.8 (± 8.3)	920.9 (± 169.9)	3.9 (± 0.6)	3.6	603.6 (± 22.2)	16 (± 2.0)	9.7
51.4 (± 8.3)	1365.7 (± 93.2)	3.7 (± 0.5)	5.1	816.2 (± 64.1)	14.3 (± 2.2)	11.6

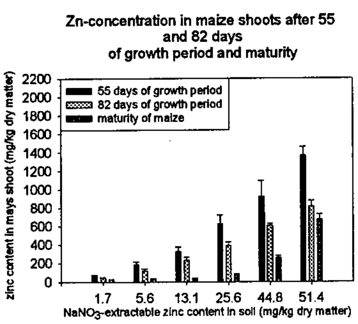

Figure 1: Effect of varying mobile zinc contents in soil on zinc concentrations in maize shoots at different growth stages

ASSESSMENT OF SOIL - PLANT RELATIONSHIP USING METHODS OF MULTIVARIATE REGRESSION

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1. Introduction

Soil pollution assessment is always connected with extending costs. Reduction of sampling sites could give us prejudiced results (FORTUNATI et al., 1994). A systematic fore-investigation of vegetation pollution (selected test plants) could be useful for a design soil sampling more rational. One important condition must be curried out - a good correlation between concentration of pollutants in plant and soil. A simple universal correlation is usually not significant because of complexity of the processes. Methods of multivariate regression offer the possibility for better characterization of soil - plant relationship.

2. Materials and Methods

Top soils (0 - 5 cm) and leaves of test plant (*Plantago lanceolata* L.) were sampled on 39 sites in Celje County, Slovenia, were soils are polluted with Zn, Cd and Pb. Soil samples were treated and usual soil parameters (pH, % of total organic matter, % of total nitrogen, available phosphorous and nitrogen compounds) were analyzed by standard chemical procedures (PAGE et al., 1982). Exchangeable and total heavy metals were determined using various techniques of atomic absorption spectrometry (ISO/DIS 11047, 1995) after extraction with 1% EDTA (URE and BERROW, 1970) or dissolution with aqua regia (ISO/DIS 11466, 1993). After drying (40°C) and grinding, plant tissue samples (250 - 500 mg d.w.) were treated with 65 % HNO_3 acid and dissolute in microwave oven. Electrothermal technique was used for the determination of Cd and Pb and flame AAS for the determination of Zn content in plant tissues (HUDNIK and KOZAK LEGISA, 1992). To characterized the relationship between plant and soil heavy metals' concentration, different regression analyses were used under STATISTCA and UNSCRAMBLER software.

3. Results and Discussion

The univariate regression between plantain metal content and soil metal concentration (aqua regia dissolution) is poor for Zn and Cd and particularly for Pb. The correlation is better if EDTA extracted metals are taken into account (Table 1).

Using methods of multivariate regression we increased multiple r (Table 1). In first case we took a priory only soil parameters that directly influenced on bioavailability of metals: pH, content of organic matter and phosphorous, and particular metal concentration (ERIKSSON, 1989). Soil parameters increased correlation coefficient for Cd and Pb, but not so obviously for Zn. The most important factors that influence on metal content in plant are soil pH value for Cd and content of

organic matter and soil pH for Pb. We could explain this fact with strongly bounded Pb on organic fraction in the soil and big influence of soil pH on desorption processes for Pb and Cd (ADRIANO, 1986; KABATA - PENDIAS and PENDIAS, 1992)

Table 1: Correlation coefficients for plant and soil - total and exchangeable metals (n = 39)

		UR	MLR - a priory			MLR - stepwise	PLSR
			M* + pH	M + pH + OM	M + pH + OM + P	all soil features	all features
Cd	aqua regia	0.52	0.59	0.63	0.64		
	EDTA	0.59	0.65	0.69	0.70	0.93	0.88
Zn	aqua regia	0.54	0.56	0.58	0.59		
	EDTA	0.68	0.69	0.72	0.73	0.97	0.99
Pb	aqua regia	0.23	0.37	0.46	0.47		
	EDTA	0.16	0.34	0.44	0.45	0.84	0.89

* particular metal concentration in soil; OM = % of organic matter in soil; P = available phosphorous in soil

Using forward stepwise multiple linear regression and partial least squares regression where also other soil and plant parameters are included into the model, the relationship between soil and plantain metal concentration become statistically significant (Table 1).

4. Conclusions

Using different methods of multivariate regression we increased correlation coefficient for plant and soil content of heavy metals. Best characterization of plant - soil relationship for Zn and Pb we get by using partial least squares regression, and for Cd by using stepwise multiple linear regression. Those relationship models could be used to assume the transfer of Cd, Zn and Pb in both directions: from plants to soil and from soil to plants.

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PLANT AVAILABILITY OF CADMIUM IN BRAZILIAN SIMPLE SUPERPHOSPHATES

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1. Introduction

Concerns have emerged recently on the possible dangers for health of cadmium (Cd) in the environment, even in low concentrations. There is conclusive evidence that Cd poses a hazard for human and animal health if its disposal is uncontrolled (Page and Bingham, 1973). Contamination of soil with Cd was shown to occur with phosphorus fertilizer (Williams & David, 1973). Information on the Cd availability for the plants from Brazilian simple superphosphates produced from different raw materials are not available, and it was the objective of this work.

2. Materials and Methods

An experiment in a greenhouse was conducted with a medium textured Red-Yellow Latosol (Typical Hapludox). The base saturation was adjusted to 70% (approximately pH 6.2) by adding dolomitic limestone. Nitrogen and potassium were added at rates of 200 and 250 mg kg^{-1} of N and K, respectively, as well as a mixture containing all micronutrients.
The experiment was conducted in a completely randomized block design with four replications. The treatments were arranged in a 5 X 2 X 3 factorial schedule, consisting of five simple superphosphates (P sources, raw material from Araxá – SS1, Jacupiranga – SS2, Catalão 1 – SS3, Catalão 2 – SS4 and Tapira – SS5) that were either leached or not in order to remove the water-soluble P (original and leached), and three methods of application (thoroughly mixed with the whole soil in the pots and with 10% and 1% of its volume).
In order to remove water-soluble P from the phosphorus sources, the procedure suggested by Bartos et al. (1992) was used. Application rate was 50 mg kg^{-1} P, calculated considering the content of P soluble in NAC + H_2O (AOAC, 1984).
Eight seeds of hybrid corn per pot were planted at a depth of 1 cm, which were thinned to three plants per pot. Deionized water was added at a rate of 170 mL kg^{-1} of air-dried soil. Nitrogen and sulfur were applied at rates of 250 mg kg^{-1} N and 50 mg kg^{-1} S split in two applications. Plant tops were removed 35 days after planting. The harvested material was dried at 50° C, weighed, and subsamples subjected to digestion in a microwave furnace. The determination of Cd was performed in a ICP-AES.

Results obtained (X) for Cd concentration and Cd uptake were transformed utilizing the formula $X^{0.5}$ and evaluated by analysis of variance, considering the factors studied as causes of variation. Results for dry matter were not transformed. Tukey test at 0.05 significance level was used for multiple comparisons.

3. Results and Discussion

The analysis of variance for dry matter yield by corn plants showed a triple interaction effect for simple superphosphates, leaching and method of application, which had to due with the P availability in the P sources studied.

The Cd concentration in the corn plants varied from 0.002 to 0.502 mg kg^{-1} and the amounts of Cd accumulated from 0.35 to 1.22 µg pot^{-1} (mean of 0.70 µg pot^{-1}).

Some differences were noticed in dry matter and Cd concentration when the effect of leaching was compared. The original P sources provided higher dry matter yield (12.2 g for the original and 5.5 g for the leached) and lower concentration of Cd in the corn plants (60 µg kg^{-1} for the original and 124 µg kg^{-1} for the leached). The uptake of Cd by the plants were not statistically different (0.71 µg pot^{-1} of Cd for the original and 0.67 µg pot^{-1} for the leached).

The methods of application affected the Cd accumulated by corn plants. Simple superphosphates, applied totally mixed with the soil, provided conditions for higher uptake of Cd than when the same P sources were applied localized with 10% and 1% of the total volume of the soil in the pot (Table 1). There were no differences in the uptake of Cd by the corn plants between the simple superphosphates studied.

Table 1. Effect of method of application of simple superphosphate on the Cd accumulation by corn plants grown for 35 days in samples of a Red-Yellow Latosol [1]

Method of Application [2]	Cd uptake
	µg pot^{-1}
Mixed	0.79 a
Localized 10%	0.66 b
Localized 1%	0.63 b

[1] Values followed by the same letter are not statistically different ($p < 5\%$)
[2] Mixed= P source thoroughly mixed with the whole soil in the pot; Localized 10%= mixed with 10% of the soil in the pot; Localized 1%= mixed with 1% of the soil in the pot.

4. Conclusions

The amounts of Cd accumulated by the corn plants were low and not different when comparing the uptake provided by the simple superphosphates produced from five different Brazilian raw materials.

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BIOMASS UPTAKE RELATED DEPLETION OF SOIL CD IN A TREE-SPECIES EXPERIMENT ON FARMLAND IN SWEDEN

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1. Introduction

During the 20th Century, concentrations of the heavy metals cadmium (Cd) in Swedish agricultural soils have increased by an average of 33 % (Andersson, 1992). In addition to efforts aimed at reducing the amounts of heavy metals in fertilisers and atmospheric deposition, it has been proposed that soils could be cleaned by allowing energy crops to take up the heavy metals and then harvesting the contaminated biomass (phytoremediation). Fast-growing trees, such as species of *Populus* and *Salix*, have proved to be effective in taking up heavy metals (Brieger *et al.*; 1992; Landberg & Greger, 1996). The aim of this study was to assess the potential of six different tree species to allocate Cd to different biomass compartments as well as to investigate changes in soil variables (pH, Cd) over the first six year period.

2. Materials and Methods

In a replicated tree species experiment in southern Sweden, including five different fast growing deciduous tree species (Crack willow, Grey alder, Hybrid aspen, Hybrid poplars and White birch), the soil was thoroughly sampled at the time of planting. Six years later both the biomass and soil was sampled. All soil samples were analysed with respect to soluble (NH_4NO_3 extract.) and total (HNO_3 extract.) Cd as well as pH and the biomass compartments of each tree species were analysed for total content of Cd.

3. Results and Discussion

A decrease in $pH_{(H2O)}$ of 0.2-0.5 units over the period was found. A high biomass uptake of Cd was not related to a corresponding depletion of the soluble soil Cd pool. However, the sum of the Cd uptake in the above-ground biomass, field vegetation and litter/O horizon was negatively correlated ($p<0.018$) with the total concentrations of Cd in the A-horizon layers between 2 cm - PD. There was also a non signficant tendency for accumulation of Cd in the uppermost 2 cm layer. A tentative budget for Cd in the ecosystem at the experimental site was based on the linear relationship found (Table 1). Assuming a biomass (including aboveground tree, field vegetation and the Litter + O) uptake of 1 or 10 mg m^{-2}, the weighted depletion in total Cd over the six year period would be 0.4 (0.36-0.38) and 11.2 - 12.1 mg m^{-2} respectively, in the 2 cm -PD layers of the A-horizon varying with soil bulk density of 1.3-1.4 Kg

dm^{-3}. Some of the "missing amounts" of Cd may have been retranslocated to the top centimetres of the soil or stored in coarse roots. A possible root pool of Cd was estimated using relations of root uptake compared to the whole biomass uptake from *Salix viminalis* studies at similar dry weight production levels as in our experiment (Labrecque *et al,* 1995). The difference between contribution and uptake/retranslocation indicate whether there has been any leaching out of the A-horizon (negative values in Table 1) or an uptake from deeper soil horizons (positive values). Due to the uncertainty in some pools included in the budget, it is difficult to get a complete understanding of whether Cd actually leached from the A horizon or if Cd was retranslocated from deeper soil horizons. However, it may primarily be concluded that soil depletion may be counterbalanced by a high biomass uptake.

Table 1. A tentative Cd budget for a 6 year old forest plantation ecosystem

	Cd mg m^{-2}	
	Biomass uptake 1 mg m^{-2}	Biomass uptake 10 mg m^{-2}
Contribution		
Soil depletion	-0.4	-11.2 to -12.1
Atmospheric deposition *)	-1.0	-1.0
Weathering	- ?	- ?
Uptake/retranslocation		
Biomass uptake 'harvestable'	0.4	6.8
Biomass uptake 'not harvestable'	0.6	3.2
Retranslocation to top 2 cm soil **)	0.1	0.9
Roots***)	0.2	1.4
Difference		
Possible leaching or accumulation in the A-horizon	-0.1	-0.8 to + 0.1

*) Atmospheric deposition of Cd to Bullstofta over 6 years as estimated from moss analysis (Rühling, 1994 ; Berg et al, 1995; Rühling et al, 1996), **) Approximated retranslocation to the 0-2 cm mineral soil from regression functions.
***) Estimated as 20 % of total biomass uptake

4. Conclusions

Over the experimental period there was a significant decrease in soil pH as a result of cessation of cultivation and tree planting. The soluble pools of Cd in the A-horizon were not depleted by the high biomass uptake, but increased somewhat as a result of a decrease in pH. However, a biomass uptake related depletion in the total concentrations in the A-horizon was found for Cd.

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MERCURY LEVELS IN NATURAL SOILS AND TWO PLANT SPECIES (*SPARTIUM JUNCEUM L.* AND *AVENA SATIVA L.)* IN LATIUM (CENTRAL ITALY).

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1. Introduction

Despite the potential hazard of Hg, relativelylittle attention has been paid to its distribution in Italian soils. Owing to past and recent volcanism and the related hydrotermal and gaseous activity, many anomalies of Hg have been recognized in Central and Southern Italy. In these areas Hg was exploited starting from prehistoric age until historic times. Mining activities released Hg at larger rates than related to natural processes causing the contamination of relatively large areas. To improve the knowledge about the background values of Hg in Italian soils, a survey was made in natural soils of Latium (central Italy). This region has been impacted for long time by intense volcanic activity. With the aim to stress the ecological impact of Hg in relation to its toxicity Hg was also determined in two plant species : *Spartium Junceum and Avena sativa*. These plants grow naturally in these soils and their distribution is rather uniform all around the region.

2. Materials and Methods

102 representative top-soil samples (0-5 cm) were collected, dried at 40°C and sieved through a 2 mm teflon coated sieve. Standard pedological analyses were performed according to U.S.D.A. methods.

Soil Hg extraction was carried out in the fraction < 0.1 mm with a mixture of ultrapure nitric and hydrocloric acid (3:1) in a microwave digestion system.

Hg in plants stem and branches was extracted with ultrapure HNO_3 ,Hg in soils and plant was determined by CVAAS, with a Perkin Elmer 5100.

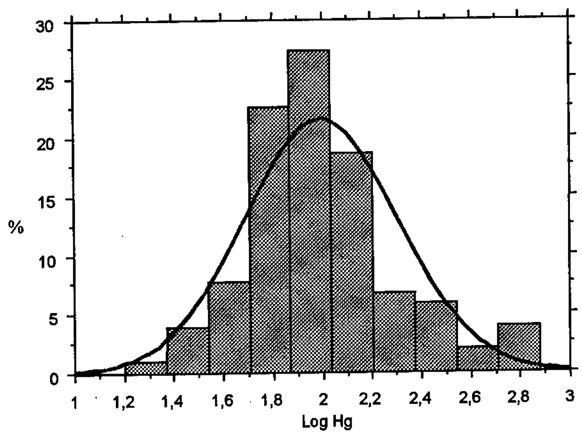
All Hg analysis were previously tested introducing international CRM.

3. Results and Discussion

Hg concentration in Latium soils ranges between 16 to 754 $\mu g\text{-}kg^{-1}$ with an average value of 135 $\mu g\text{-}kg^{-1}$. Soils developed on volcanic rocks are signigicantly richer in Hg if compared with soils on sedimentary rocks, mean content are 166 $\mu g\text{-}kg^{-1}$ and 108 $\mu g\text{-}kg^{-1}$ respectively.

The frequency distribution of Log Hg; is reported in Figure 1. A comparison with data of (Schlüter.1993) shows that our data are generally close to 'normal' Hg concentration of top-soils. Data from the correlation matrix betwen Hg and some pedological variables show that only organic matter is significtively correlated.

Figure1. Frequency distribution of LogHg in Latium soils (μg/kg)



Range=1,996
Std. Dev=0,311
E.Std.=0,031
n° of samples=102
Min =1,2
Max =2,88
Variance=0,097
Coeff.of Var.=0.156
Geom. mean=1,973
Skewness=0,733
Kurtosid=1,065
Mediane=1,945

Hg concentrations in the two plant species are: 48±21.8 μg-kg^{-1} and 40±17.2 μg-kg^{-1} for *Avena* and for *Spartium*. These close values of Hg results to be irrespective of the ecological and morphological differences between the two plant species.
The difficulty of soil Hg to translocate to plant is also evidenced by the concentration ratio, that was generally <1 with the exclusion of only one sample (1.42).

4. Conclusions

Hg in Latium natural soils is generally related with the parent material and the background level is comparable with other data from literature despite of the high geochemical anomalies of this area. Soil Hg is strictly related to O.M. and a relevant role has been also observed for pH that, in soil with a pH close to neutrality, as most of the studied soils, enhances the retention of inorganic Hg by iron oxides and clay minerals.
Spartium and *Avena* show a very similar Hg content. The concentration ratio below 1 show the difficulty of soil Hg to be transferred to plants. In fact as is well known the accumulation of Hg in plants is mainly related to the processes of foliar uptake. This could explain the scarce relation found between Hg in soil and in plant.
In fact the ready available Hg for plants is mainly Hg° that is present in the environment as gaseous Hg as a consequence of soil degasing processes.

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UPTAKE AND DISTRIBUTION OF CADMIUM IN CUCUMBER PLANT AS AFFECTED BY NITROGEN FORM

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1. Introduction

Cadmium is one of the most toxic heavy metals that usually are found in polluted environments. The main sources of Cd found in crop fields are due to the use industrial products (such as electronics), phosphate fertilizers and municipal waste compost. Toxic heavy metals are of interest due to their tendency to accumulate in soils and to be taken up by plants. High uptake of cadmium by plants may consequently increase the diet intake by humans, who consume these plants as food source (Yantiang Guo,1995). The nitrogen forms play a dominant role in the uptake of nutrients and in the resulting cation/anion uptake balance. The aim of the present study was to assess the influence of the nitrogen form on the uptake and distribution of Cd by cucumber plant grown in nutrient solution at controlled pH.

2. Materials and Methods

The experiment was conducted under green house conditions with cucumber (Cucumis sativus L.) in hydroponics culture. Plants were grown on aerated complete nutrient solution with NH4 or NO3 as the forms of N nutrition (Table,1).

Table (1) Chemical composition of the basal nutrient solutions (mol / m3)

Form of N	K	Ca	Mg	NH4	NO3	H2PO4	SO4
NH4	6.0	5.0	2.0	15.0	-	1.0	17.0
NO3	6.0	5.0	2.0	-	15.0	1.0	2.0
Micronutrients (mg L-1): B 0.5; Fe 0.5; Mn0.5; Zn 0.05; Cu 0.02. and Mo 0.01.							

Different rates of Cd 0.5, 1, 3, 5 and 10 $mg.L^{-1}$ were added to the nutrient solution as CdCl2. Each treatment was in triplicate and every replicate included two plants per pot. Cd was added to each of the following solution renewals. Plants were harvested after 35 days exposure to Cd treatments. The various plant parts (edible part, shoots and roots) were separated and oven dried at 70°C. Concentration of Cd, Zn and N in the various parts of plants were determined according to Cottenie et al. (1982).The randomized complete block design was done according to Gomez and Gomez (1984).

3. RESULTS AND DISCUSSION

The dry matter yield of both N forms was significantly reduced by increasing Cd levels in the nutrient solution. Plants grown on NO_3 had higher dry weights as compared to NH_4 even even when Cd levels were high.The nitrogen uptake significantly decreased as the Cd level increased in plants grown on NH_4 or NO_3 nutrient solution. The Cd- concentration in shoots and roots of plants grown on NH_4 were significantly higher than in plants grown on NO_3. The distribution of Cd in the cucumber plant was independent of the form of N. Cd uptake by plants was significantly increased with increasing Cd concentration in the nutrient solution because of the increasing rate of absorption Cd than the decreasing rate of accumulation of the dry matter (concentration effect).

It is also noticed that the Cd uptake by plants grown on NO_3 solution was higher compared to plants grown on NH4. From these observation we conclude that the form of N nutrition has a direct effect on Cd uptake by cucumber roots.

The shoot/root distribution of Cd was roughly similar for NH_4 and NO_3-grown plants. A comparison of the Cd distribution of both root and edible parts showed a much lower proportion in edible parts as compared to the roots. About 65% of the Cd taken up by plants grown on NO_3 with 0.5, 1.0 and 3.0 ppm Cd in solution accumulated a smaller amount (about 2.3%) of Cd taken up as compared with in the shoot (18.35% relative to the whole plant).

4. Conclusion

The Cd concentrations in NH4 grown were higher as compared to plants grown on NO_3. The uptake Cd was distributed in the plant independent of N form. This shows that the N source directly affects the availability of Cd for uptake by roots.

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TRACE ELEMENTS UPTAKE BY WHEAT GRAINS DEPENDING ON SOIL TYPES (QUASAR PROGRAMME)

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1. Introduction

An increasing concern is world-wide observed about soil and crop quality, special attention being paid on potentially harmful compounds such as trace elements. It is a public health issue and a stake in international trading as well. That is why INRA has launched a research programme called QUASAR ("QUAlité des Sols Agricoles et des Récoltes").

Aims

This programme had two aims: first to determine to what extent soil properties can affect the trace elements concentrations in wheat grain; second, to establish indisputable reference values in the frame of "usual" agriculture i.e. without spreading of sewage sludge or other wastes. This allows thereafter to demonstrate whether sewage sludge spreadings on agricultural land noticeably affect the wheat grain composition.

Sampling strategy

For ruling out the influence of the variety, only one cultivar of winter wheat was sampled (i.e. Trémie) and the sampling scheme was based on soil series.(MENCH et al., 1997).Ten soil series located in various natural regions of the northern half of France were selected (table 1). For each soil series, 11 to 15 sites in rather scattered parcels have been investigated. A thorough inquiry was carried out on each site regarding the cultural practices during the last ten years, which can provide useful information for subsequent data interpretation. Numerous collaborations have been very helpful for selecting the 128 parcels, for investigating the cultural practices, and for sampling ears just before harvest.

2. Materials and Methods

Soil and wheat sampling

The wheat ears were sampled from 1m² plots. Grain was mechanically husked, special attention being paid to limit contamination. Grain fresh weight and moisture were determined. This allows us to assess fluxes per hectare. Grains were washed, oven-dried and milled in a Ti-surfaced grinder. The surface ploughed soil horizons were exactly sampled at the place of grain sampling. Peds were crushed by hand in a porcelain mortar then passed through a 2 mm sieve.

Selected soil series

Among the selected ten soil series (table 1), the "chestnut coloured earths" developed over the Sinemurian limestones are known as containing high geogenic concentrations of trace elements (BAIZE et CHRÉTIEN, 1994; BAIZE, 1997; MENCH et al. 1997).

Soil and grain analyses :

Analyses of the topsoil : usual analyses of characterization + total content in Ca, Cd, Cu, Cr, Fe, K, Mg, Mn, Ni, P, Pb and Zn. Digestion by HF and $HClO_4$. Tl content : digestion by HNO_3 and H_2O_2. Extractable Cd, Pb and Zn by using four chemical reagents : $NaNO_3$ 0.1 M and 0.01 M, $CaNO_3$ and $CaCl_2$. Assessment of the physico-chemical availability of cadmium by the isotopic exchange kinetic technique. Grain analyses : total content in Ca, Cd, Cu, Cr, Fe, K, Mg, Mn, N, Ni, P, Pb, Tl, Zn. Wet digestion in nitric acid and hydrogen peroxide.

Table 1 : *Short description of the ten soil series studied.*

A	silty-sandy soils, with a natural trend to high acidity, over old alluvial terraces (Sologne)
B	thick, neutral, silty-clay-loam developed in a loess-like deposit over limestone (Grande Beauce)
C	non calcareous soils developed in residual clays over Jurassic limestones ("Terres d'Aubues" - Basse Bourgogne)
D	neutral, thick, silty-clay loam developed in an iron-rich residual clay formation over Sinemurian limestones ("chestnut coloured earths" - Auxois)
E	slightly acid Luvisols Dégradés, silty at the surface, derived from an old loess-like deposit (Gâtine Tourangelle and Boischaut nord)
F	neutral Néoluvisols, silty at the surface, derived from loess over chalk (Santerre and Vermandois)
G	neutral, thick ferrallitic palaeosols over Dogger limestones ("red earths" - Poitou)
H	strongly calcareous, shallow soils from Senonian chalk (Champagne crayeuse)
J	stony, clayey soils over Oxfordian limestones (Champagne Berrichonne)
K	calcareous, not very thick soils over Kimmeridgian marls and limestones (Aunis)

3. First results

To date, all soil data are available, but only some grain analyses. The 128 Cd values in grains range between 0.015 and 0.165 $mg.kg^{-1}$ (median = 0.045) while the Zn values range from 7.0 to 43.0 $mg.kg^{-1}$ (median = 15.5). Table 2 shows that Cd and Zn concentrations in grains vary widely within each soil series but also from one to another. Their order of decreasing concentration is markedly different for Cd and for Zn. The soils of the J series producing the richest grains in Cd exhibit high concentrations in Cd (0.58 to 1.33 $mg.kg^{-1}$ - median = 0.80). On the other hand, the A series (growing wheat grains rich in Zn) corresponds to silty-sandy siliceous soils, with low natural background levels in trace elements.

Table 2 : *Cd and Zn concentrations in wheat grains (expressed as $mg.kg^{-1}$). The soil series are sorted by decreasing medians.*

	Cd				Zn		
Series	**1st dec.**	**median**	**9th dec.**	**Series**	**1st dec.**	**median**	**9th dec.**
J	0.064	**0.088**	0.112	**A**	18.2	**23.5**	32.8
H	0.033	**0.061**	0.093	**D**	12.5	**22.6**	25.3
C	0.027	**0.055**	0.098	**H**	14.5	**18.9**	23.1
A	0.036	**0.053**	0.112	**G**	14.9	**17.3**	22.1
B	0.036	**0.050**	0.084	**C**	10.8	**15.3**	19.3
D	0.029	**0.044**	0.053	**F**	10.2	**15.3**	18.5
F	0.029	**0.043**	0.051	**K**	12.4	**15.2**	22.1
K	0.016	**0.040**	0.047	**E**	10.3	**14.1**	16.5
E	0.023	**0.032**	0.097	**J**	10.7	**13.2**	15.9
G	0.024	**0.032**	0.067	**B**	08.9	**12.3**	13.7

4. Conclusions

Most of our expected results are very late. It seems however that each soil series, by its own set of properties, can determine the concentrations of certain trace elements in wheat grain. Such a fact cannot be disregarded in the future.

More results will be presented on the poster.

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PARAMETERS OF HEAVY METALS BIOAVAILABILITY IN SOIL

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1. Introduction

Heavy metal pollution is very frequent kind of soil degradation located mainly inside of urban or industrial areas. Many soil parameters are influencing mobility (bioavailability) of heavy metals in soil, like pH, humus content, content of carbonates, soil texture, etc. The uptake of heavy metals by plants is sensitive not only from soil parameters but also from plant ´s characteristics. Therefore the correlations between heavy metal contents in soil and contamination of plants is not high significant everywhere and everytime.

2. Materials and Methods

Soil samples were taken out from 119 different polluted soils (from the depth 0-0.2 m). Relevant cultivated plants were sampled simultaneously. Both soil and plant samples were air dried and after it homogenized. Soil extractions were carried out by 2M HNO_3, 0.05 M EDTA, 0.01 M $CaCl_2$, 1 M NH_4NO_3 and by mixture of acid (HF + HNO_3 + HCl). Heavy metals from plant tissues were extracted by concentraced HNO_3 (microwave digestion). Cd, Pb, Zn, Cr, Co, Cu, Ni were determined in both soil and plant samples by methods of FAAS and GAAS.

3. Results and Discussion

Different soil extractions led to different extracted quantities. More intensive extraction was found out when HNO_3 and EDTA extractant were used in comparison to NH_4NO_3 and $CaCl_2$ extractions. In case of less effective extractions following order of extraction capacities we can identify: Cd>Zn>Ni>Cu>Pb>Co>Cr. When stronger agents were used it is: Cd>Pb>Cu>Zn>Ni>Co>Cr.
Bioavailability of heavy metal in soil and its uptake by plants are depended on many parameters of soil and on kind of plant. pH carbonate content, organic mater content (and quality too), soil texture, cation exchange capacity, and other parameters could be those soil properties which decide about mobility of heavy metals in soil and their ability to move from soil into the plant (Brümmer, 1986; Alloway, Ayres, 1993; Weissenhorn et al., 1995; Wenzel, Blum, 1995, and others).
From our results highest bioavailability of heavy metals in soil was generally identified in light soils. pH range from 5.5 to 6.5 is most favourable for heavy metals extractions from soil by all tested extractants. When humus content is decreased heavy metals bioavailability is increased.
Most significant correlations between soil and plant contents of heavy metals were found out in case of Cd and Zn. In oposite no correlations we derived when Cr, Cu, Co, Ni and Pb was evaluated. Of coarse it is depended also on extractants. Generaly we can say that more effective correlations were determinned after EDTA, HNO_3 soil extractions. It was visible mainly in case of Cd and Zn contents.

4. Conclusions

Is very difficult to say what extractant is most appropriate for soil pollution and for relevant risk assessments of plant contamination. Results from this work could motivate to prefer EDTA and HNO_3 as extractants when we want to have usable data for forecast of crop contamination. It is most acceptable for Cd and Zn, less for Cu, Pb and no acceptable for Co and Cr.

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EFFECT OF LIME ON CD AND PB UPTAKE BY SUDANGRASS

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1. Introduction

Contaminant metals such as Cd and Pb can often accumulate in the plant tissue and exceed the levels that are toxic to man or animals before they produce visible phytotoxic effects. Immobilising the metals in the soils can be a solution to minimise plant uptake (KABATA PENDIAS and PENDIAS, 1992). Soil pH is known to influence the retention in soils and the plant availability of a number of trace elements such as heavy metals (CORDOVIL et al., 1997; SPARROW and SALARDINI, 1997). Nevertheless, there are contradictory effects of liming, which require further study (HAN and LEE, 1996). The main objective of this study was to assess the effect of liming on sudangrass growth and the uptake of Cd, Pb and other elements.

2. Materials and Methods

An acid silt loam soil with the following characteristics, was used in this experiment: soil pH (H_2O) - 5.3, organic matter - 0.94 %, total Pb - 8.63 mg kg^{-1} and total Cd content 0.09 mg kg^{-1}. After dried and sieved (2 mm mesh), soil was limed with 0 (A), 1.5 (B), 3.0 (C) or 4.5 (D) g $CaCO_3$ kg^{-1}, and incubated 2 weeks at 70% field capacity. Then pots were treated with 0 (0Pb), 50 (1Pb) and 500 mg (2Pb) Pb kg^{-1} ($(CH_3COO)_2Pb.3H_2O$) or 0 (0Cd), 1 (1Cd) and 10 mg (2Cd) Cd kg^{-1} ($3CdSO_4.8H_2O$) and again incubated 4 weeks at 70% field capacity. After incubation, N, P, K, S, and micronutrients were added at adequate rates for plant growth. Fifteen plants of sudangrass (*Sorghum sudanense* (Piper) Stapf) cv. Tama per pot (1.5 kg soil) were grown with three replicates. After one month they were harvested. Total elements in the plants were extracted by concentrated HNO_3 and Pb and Cd determined by ASV (NETO and VARENNES, 1993) and Ca, Mg, Fe, Cu, Zn and Mn determined by AAS (Atomic Absorption spectrophometer).

3. Results and Discussion

Lime application increased the soil pH (H_2O) from 5.2 to 5.7, 6.3 and 6.9 respectively of B, C and D liming levels, regardless of the metal applied. Although there were no differences among B, C and D rates, stimulation of the growth of sudangrass by lime was observed even at the lowest level, being more evident on Pb treatments (Table 1). Negative effects of Cd and Pb on sudangrass, seem to diminish drastically at pH 5.7 and higher. Apart from the effect of higher pH, this could also have been the result of a better calcium nutrition, regardless of the metal present. Liming the soil, stimulated in all cases the absorption of Ca (data not shown). Not only Ca was added to the soil by means of CaCO3 but also heavy metals may compete with Ca for the adsorption sites, mobilising more Ca into the soil solution. Plant growth was affected by Cd amendment, within the same liming treatment, (A, B, C and D), and by Pb addition only when the soil was not limed (Table 1). Liming generally decreased Pb and Cd content in plant shoots (Table 2). This effect of liming on plant metals content was more significant for Pb. In fact, Pb is relatively less easily taken up by plants than Cd, because Pb has lower mobility and is less easily translocated into plants (HAN and LEE, 1996).

Table 1: Mean dry weights (g) of plants cultivated on the different treatments.

Liming level	0 Pb	1 Pb	2 Pb	0 Cd	1 Cd	2 Cd
A	6.3 (c, 1)	7.4 (c, 1)	4.9 (b, 2)	6.3 (c, 1)	5.9 (b, 1)	3.9 (c, 2)
B	10.1 (b, 1)	9.9 (b, 1)	10.9 (a, 1)	10.1 (b, 1)	8.5 (a, 2)	8.8 (a, 2)
C	12.0 (a, 1)	10.0 (a, 1)	10.3 (a, 1)	12.0 (a, 1)	8.4 (a, 2)	6.8 (b, 3)
D	13.2 (a, 1)	10.0 (a, 1)	10.3 (a, 1)	13.2 (a, 1)	8.6 (a, 2)	6.3 (b, 3)

Values in the same column followed by the same letter, and in the same row followed by the same number, are not significantly different by to the Scheffe-F test ($p<0.05$).

Table 2: Pb and Cd content (μg/g) and uptake (g/pot) by plants cultivated on the different treatments.

Liming level	0 Pb	1 Pb	2 Pb	0 Pb	1 Pb	2 Pb
A	2.0 (a, 3)	20.2 (a, 2)	190.1 (a, 1)	12.6 (a, 3)	149.5 (a, 2)	931.5 (a, 1)
B	0.6 (b, 3)	3.7 (b, 2)	48.6 (b, 1)	6.1 (b, 3)	36.6 (b, 2)	529.7 (b, 1)
C	0.5 (b, 3)	3.0 (c, 2)	38.1 (c, 1)	6.0 (b, 2)	30.0 (b, 1)	392.4 (c, 1)
D	0.4 (b, 3)	2.2 (d, 2)	25.8 (d, 1)	5.3 (c, 2)	22.0 (c, 1)	265.7 (d, 1)
Liming level	**0 Cd**	**1 Cd**	**2 Cd**	**0 Cd**	**1 Cd**	**2 Cd**
A	1.9 (a, 3)	26.9 (a, 2)	119.8 (a, 1)	12.0 (a, 3)	158.7 (b, 2)	467.2 (b, 1)
B	0.8 (b, 3)	23.9 (a, 2)	68.5 (b, 1)	8.1 (b, 3)	203. (a, 2)	602.8 (a, 1)
C	0.2 (c, 3)	17.8 (b, 2)	53.1 (c, 1)	2.4 (c, 3)	149.5 (b, 2)	361.1 (c, 1)
D	0.08 (d, 3)	10.9 (c, 2)	47.7 (d, 1)	1.1 (d, 3)	93.7 (c, 2)	300.5 (c, 1)

Values in the same column followed by the same letter, and in the same row followed by the same number, are not significantly different by to the Scheffe-F test ($p<0.05$).

Liming significantly decreased the uptake of Pb and Cd (Table 2). The exception was the higher Cd uptake by plants grown on treatment B, that was probably due to the greater dry weight production. Reduction on plant Pb and Cd uptake resulted from decreased availability of these metals in the soil. Higher pH increases negative charges on the soil surface and reduces competing cations, thus increasing adsorption sites. A Ca ion competition for translocation can also be considered as a factor influencing shoot uptake of the metals (CORDOVIL et al., 1997). Liming the soil, did not significantly influence Mg, Fe or Cu concentration in sudangrass shoots. However, applying $CaCO3$ to the soil greatly decreased Zn and Mn content in plants (data not shown) regardless of the level of Pb or Cd added to the soil. Heavy metals can be antagonistic to Zn and Mn, and an adsorption competition between Ca and these microelements is also possible.

4. Conclusions

Liming the soil increased the soil pH from 5.3 to 5.8, 6.4 and 6.8. As a result of increase in pH, plant growth was significantly enhanced relative to the unlimed soils. At the lowest liming rate (pH 5.7), a positive effect on plant growth was observed. Liming generally decreased Pb and Cd absorption and their translocation to plant shoots.

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INFLUENCE OF SOIL PH CHANGE ON SOIL AAAC-EDTA EXTRACTABLE ZINC, FOLIAR ZINC CONCENTRATION, AND ZINC UPTAKE BY CLOVER PLANTS

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1. Introduction

The soil pH has an important role in the bioavailability of zinc, as this decreases with an increase in soil pH (4). Soil extraction with the reagent 0.5M NH_4OAc + 0.5M HOAc + 0.02 M EDTA (AAAc-EDTA) solution at pH 4.65 is mainly used to predict the Zn bioavailability in soils with low pH (3), as it simulates the uptake of the element from the soil solution by the plants. Under an alkaline industrial residue application, soils pH was highly influenced (1,2). The main aim of this study was to relate soil pH to plant Zn parameters, such as foliar Zn concentration and Zn uptake, after liming some soils with the residue. Also, the relationships between AAAc-EDTA extractable Zn and soil pH, foliar Zn concentration, and Zn uptake were investigated.

2. Materials and Methods

Seven topsoils (0-20 cm), belonging to the soil units Aric Antrosol (ATa), Dystric Cambisol (CMd), Dystric Leptosol (LPd), Gleyic Podzol (PZg), Humic Alisol (ALu), Humic Cambisol (CMu), and Umbric Fluvisol (FLu), were studied in a pot experiment already described (2), with four industrial alkaline residue application rates (L_0, L_1, L_2, and L_3) and three replications. Subterranean clover cv. Seaton Park was used as test plant. Results for AAAc-EDTA extractable Zn, soil final pH, foliar Zn concentration, and Zn uptake were analysed by parewise correlations.

3. Results and Discussion

Tables 1, 2, 3, and 4 present AAAc-EDTA extractable Zn concentrations, soil final pH values, foliar Zn concentration, and Zn uptake. A summary of the correlations found for AAAc-EDTA extractable Zn, soil final pH, foliar Zn concentration, and Zn uptake is shown in Table 5.

Table 1 AAAc-EDTA extractable Zn (mg kg^{-1}), averaged over replicates

	Soil unit						
Residue rates	PZg	CMd	ATa	CMu	ALu	LPd	FLu
L_0	2.10	1.70	1.70	3.60	5.75	1.75	17.5
L_1	1.40	1.65	1.85	2.25	4.60	2.00	11.9
L_2	1.45	0.80	1.20	1.95	2.55	1.20	11.2
L_3	1.75	0.60	1.10	1.20	1.85	1.30	4.80

Table 2 Soil final pH, averaged over replicates

	Soil unit						
Residue rates	PZg	CMd	ATa	CMu	ALu	LPd	FLu
L_0	4.15	4.32	4.27	4.07	4.12	4.28	4.23
L_1	4.83	4.73	5.33	4.66	4.83	4.70	4.90
L_2	5.23	5.13	5.50	5.35	5.57	5.10	5.67
L_3	5.60	5.67	6.15	5.95	6.05	5.65	5.68

Table 3 Foliar Zn concentration (mg kg^{-1}), averaged over replicates

Residue rates	Soil unit						
	PZg	CMd	ATa	CMu	ALu	LPd	FLu
L_0	608.57	415.21	236.09	487.54	502.48	352.82	690.54
L_1	301.63	174.73	186.84	200.04	135.24	194.20	268.48
L_2	200.16	121.73	103.56	188.96	85.14	140.47	137.92
L_3	138.80	64.29	52.34	120.07	63.00	103.00	91.00

Table 4 Zn uptake (mg/pot), averaged over replicates

Residue rates	Soil unit						
	PZg	CMd	ATa	CMu	ALu	LPd	FLu
L_0	2.328	2.202	2.445	0.749	2.018	1.278	6.036
L_1	1.855	1.327	2.279	1.531	1.472	1.191	3.108
L_2	1.564	1.103	1.704	1.315	1.376	1.082	1.885
L_3	1.344	0.616	0.729	0.726	0.692	0.969	1.943

Table 5 Parewise correlation found for AAAc-EDTA extractable Zn, soil final pH, foliar Zn concentration, and Zn uptake

Parewise comparison	Correlation	Significance level
AAAc-EDTA extractable Zn *vs.* soil final pH	-0.232	n.s.
AAAc-EDTA extractable Zn *vs.* foliar Zn	0.465	*
AAAc-EDTA extractable Zn *vs.* Zn uptake	0.738	***
Soil final pH *vs.* foliar Zn	-0.827	***
Soil final pH *vs.* Zn uptake	-0.493	**
Foliar Zn *vs.* Zn uptake	0.615	***

* significantly different at $P\leq0.05$, ** significantly different at $P\leq0.01$; *** significantly different at $P\leq0.001$;
n.s. not significant.

There was an high significant correlation between foliar Zn and Zn uptake, which is probably explained by the high correlation observed between soil final pH and both foliar Zn and Zn uptake. The lack of a significant correlation between AAAc-EDTA extractable Zn and soil final pH explains the low correlation (although significant) between AAAc-EDTA extractable Zn and foliar Zn concentration. Between AAAc-EDTA extractable Zn and Zn uptake there was also an high significant correlation.

4. Conclusions

The correlations between soil final pH and both foliar Zn and Zn uptake indicated that these plant parameters decreased with soil pH when the residue was applied. The decreases in Zn concentration as pH rised were beneficial as regards clover nutrition, due to the relatively high foliar concentrations at the L_0 residue rate.

5. Acknowledgements
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INFLUENCE OF CHROMIUM ON THE UPTAKE OF MN, AND FE BY RADISH (*RAPHANUS SATIVUS*)

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1. Introduction

As chromium (Cr) is a redox reactive metal, its toxicity and mobility depends on its oxidation state. Within the ranges of redox potentials and pH commonly found in soils, the most important Cr oxidation states are Cr(III) and Cr(VI). Soil chemical interactions of trace elements can be important in determining resultant plant composition, and yield and deficiency symptoms might also be indicative of nutritional interactions occurring either within the plant or in the surrounding soil (GROVE and ELLIS, 1980). Cr application has been shown to affect plant Mn concentrations and uptake, and Fe deficiency symptoms have been associated with Cr toxicity (TURN and RUST, 1971; GROVE and ELLIS, 1980); conversely, OTABBONG (1989b) did not find any consistent relationship between the uptake of Mn, Cu, Zn and Fe and the magnitude of Cr toxicity, in ryegrass. This study investigates the influence of a wide range of Cr application rates in two states of oxidation on the uptake of Mn and Fe, by radish *(Raphanus sativus)*.

2. Materials and Methods

A pot experiment using radish in a Ferric Lixisol (LXf) and a Stagnic Luvisol (LVj) was carried out in a greenhouse. The experiment and some soil properties were described by FERNANDES et al. (1998). Cr was added at four rates (0, 10, 50, and 100 mg kg^{-1}), as $CrCl_3$ and $K_2Cr_2O_7$. Plant samples were digested with 65% HNO_3 and 30% H_2O_2 and plant Mn, and Fe concentrations were determined by A.A.S. Statistics was performed using ANOVA and regression analysis.

3. Results and Discussion

Different response patterns of Mn and Fe uptake to increasing levels of applied Cr were found, according to the type of soil and Cr oxidation form (Fig.1). In LXf soil, the Mn uptake increased linearly with increasing levels of applied Cr(III), reaching a maximum level of 1.65 and 0.194 mg/pot (shoots and roots, respectively) at 100 mg.kg^{-1} of Cr(III). This is mainly due to the high concentration levels of Mn in shoots and roots (3364 and 437 mg kg^{-1}, respectively) at 100 mg kg^{-1} of Cr(III) and to the related significant decrease of DM accumulation (Fernandes et al., 1998). Regarding Cr(VI), no significant effect was found on Mn uptake but a decreasing tendency was observed due to the lowering effect of increasing rates of Cr(VI) on DM accumulation, particularly in roots (ranging from 0.91 to 0.23g/pot). In LVj soil, Mn uptake was less affected by the application of Cr(III) than by Cr(VI), although showing the same pattern. In roots there was a significant effect of increasing rates of applied Cr specially from 50 to 100 mg kg^{-1}. This was mainly related to a significant increase of Cr absorption leading to a significant decrease of DM accumulation (ranging from 1.05 to 0.06 g/pot). In shoots, a similar but less relevant behavior was found with Cr(III) application, and no significant effects were found regarding the application of Cr(VI). The obtained results are in agreement with those reported by OTABBONG (1989a).

The addition of increasing rates of Cr(III) and Cr(VI) to the soils significantly lowered the Fe uptake by radish, particularly by roots. A relevant decrease was found when 100 mg kg^{-1} of Cr(VI) was added to the soils, reaching values less than 0.10 and 0.02 mg/pot of Fe in LXf and LVj soils, respectively. This is in accordance with the significant decreasing effect of Cr(VI) application on DM accumulation (ranging from 1.2 to 0.062 g/pot). Concerning shoots, no consistent relationship between Fe uptake and the application rates of Cr(VI) could be found in both soils. However, in LXf soil, the observed tendency to depress Fe uptake by Cr(VI) is probably due to the related decrease of DM accumulation (ranging from 0.91 to 0.24 g/pot). These results are in agreement with the findings of TURNER and RUST (1971).

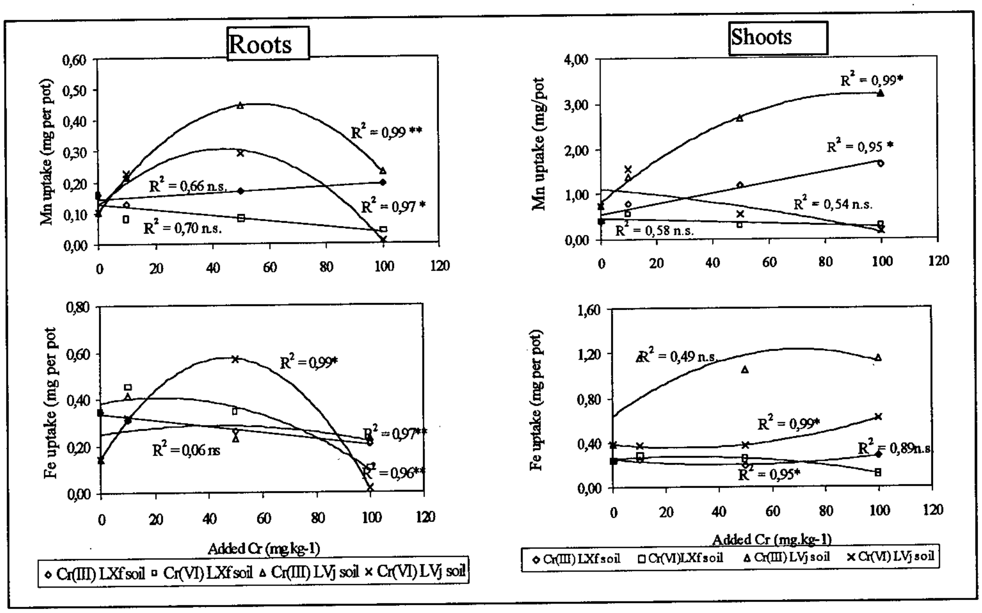

Figure 1 - Effect of two Cr oxidation forms on Mn and Fe uptake by radish in two different soils

4. Conclusions

Different behavior was found regarding Mn and Fe uptake according to the type of soil and oxidation state of applied Cr; roots uptake were more affected by Cr application than shoots, particularly by Cr(VI), in both soils; at the same application level of Cr, the uptake of Mn and Fe was more affected by Cr(VI).

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THE EFFECT OF PLANT RESIDUES ON TRACE ELEMENTS' BIOAVAILABILITY FROM CONTAMINATED SOILS

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1. Introduction

A crop response to toxicity of heavy metals depends on many factors both related to soil properties, plant (species, variety, organ and age) and also to the soil management system (tillage, crop rotation, organic and inorganic fertilization). Among factors determining heavy metals bioavailability agrotechnical factors are the least recognized so far (GRZEBISZ and POTARZYCKI, 1996; YIN-MEI ZHU and MARTENS, 1991).

The main objective of this work was to assess the dynamics of heavy metals uptake by spring triticale grown in two soils amended with plant residues of different C: N ratio.

2. Materials and Methods

A 2 x 4 factorial microplot experiment with four replications was conducted to determine how heavy metals bioavailability of two contaminated soils (loamy sand and sandy loam) is impacted by plant residues (control – without residues application, sugar beet leaves, lupines shoots and winter wheat straw). The C:N ratio was 10, 26 and 75 respectively. Soils used in the experiment were taken from an area adjacent to the Glogow Copper Smelter, Poland. Plant residues were incorporated into soil in the Autumn 1996. Spring wheat (*Triticum vulgare* L.) was used as a bioassay. Seeds were sown in spring 1997 into microplots of 0,25 m^2. Plants were harvested at three stages of wheat growth, i.e., tillering, flowering and full maturity. Metals were determined by the AAS method in dry ashed plant samples (at 540°C).

3. Results and Discussion

Total content of heavy metals assessed by means of 2 M HNO_3 test (GUPTA and HAENI, 1989) exceeded the threshold values only in the case of copper and lead (Table 1). The amount of easily available copper as determined by the 0,1 M $NaNO_3$ (GUPTA and HAENI, 1989) test at any sampling time was affected both by soil features and organic amendments. The general feature of its behavior was a progressive decrease from the time of sowing to the stage of wheat maturity.

Table 1. Chemical features of investigated soils

Soil features	LOAMY SAND	SANDY LOAM
Organic carbon, %	1.60	1.27
pH	7.7	7.5
TOTAL HEAVY METAL CONTENT, MG/KG SOIL		
Cadmium	0.15	0.18
Copper	166.8	300.0
Lead	68.0	117.0
Zinc	110.4	97.0

Higher amounts of this form of copper also occurred in loamy sand, especially in plots enriched with easily mineralizable plant residues. The uptake of all investigated metals was highly related with wheat growth stages and two distinct patterns were found. The first one refers to copper. Its uptake by wheat from control plots reached the highest values at the time of anthesis. But the incorporated plant residues were the reason of extending Cu uptake up to full maturity of wheat. The effect of soil on Cu uptake was the highest at tillering. At all growth stages Cu uptake was affected by easily mineralizable leaves of sugar beet and at full maturity also by lupines (Table 2).

Table 2. Copper uptake by spring wheat at various growth stages (mg/m^2)

Treatments	TILLERING		ANTHESIS		FULL MATURITY	
	1	2	1	2	1	2
Control	2.6	4.1	35.2	35.7	36.6	43.0
Sugar beet leaves	7.2	6.5	51.8	46.5	66.3	64.7
Lupinus	6.2	4.3	33.3	29.6	72.0	43.2
Wheat straw	5.0	3.6	30.8	29.6	50.4	47.0
LSD 0.05	0.9		7.1		10.4	

1 – loamy sand; 2 – sandy loam

The pattern of other metals uptake was slightly different of those found for copper as it is presented in Table 3 for lead. The highest amount of this metal in the shoots was found at fully matured plants. The interaction of soils and plant residues were stressed both at tillering and full maturity. However only at tillering sugar beet leaves significantly increased lead uptake by wheat in comparison with all other treatments.

Table 3. Lead uptake by spring wheat at various growth stages (mg/m^2)

Treatments	TILLERING		ANTHESIS		FULL MATURITY	
	1	2	1	2	1	2
Control	1.4	0.7	5.0	5.7	25.9	14.9
Sugar beet leaves	0.9	2.6	5.7	6.0	28.3	26.2
Lupinus	0.9	1.1	5.6	5.4	23.3	25.1
Wheat straw	0.7	1.1	5.2	5.6	19.7	23.2
LSD 0.05	0.3		-		4.4	

1– loamy sand; 2 – sandy loam

4. Conclusions

The short-term effect of organic amendments on trace elements bioavailability might be an important factor determining their entrance in to the food-chain. For areas threatened by elevated concentration of trace metals in the soil special management systems should be designed in order to diminish their mobility.

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HEAVY METAL CONCENTRATION IN WHEAT AND SOILS OF THE TALAS AND NARYN VALLIES OF KIRGISSTAN

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1. Introduction

Increasing effort is now devoted to evaluating the agricultural productivity of the soils of Kirgisstan. Although it is generally evident that the agricultural productivity is currently low, little is known about the fertility problem of soils (Karabajew et al.,1997). This is especially valid for the level of trace elements in soils and crops, eventhough it has been speculated that metal mining and coal burning in brick factories may be a source of environmental pollution with heavy metals such as Cd, Pb and Zn. In order to clarify these relationships in a German-Kirgisian joint project, a first exploratory investigation was started in 1996.

2. Materials and Methods

Seven field sites in Talas and Naryn river vallies were selected among the local agricultural communities to cover a range of soil fertility parameters (Fig. 1). The distance from potential heavy metal pollution sources was also taken into account (Table 1). After harvesting the wheat crop, during August 1996 the topsoils were sampled by auger to 30 cm depth. From the same sites representative plant samples were taken for chemical analysis. After aqua regia digestion, heavy metal concentrations were determined by atomic absorption spectrometry using a graphic furnace for Cd and Pb.

3. Results and Discussion

There are clear differences between locations with respect to trace element contents in soils and their transfer to wheat (Table 2). This variability was especially high for Pb and Cd. Soil Cd varied between 0.08 and 0.86 $mg \cdot kg^{-1}$ by a factor of 11 while soil Pb varied by a factor of 7. The variability of soil Zn by a factor of 2 was much lower.

Maximum soil concentrations for Cd and Pb were found in the sites T-4 and N-5, both of them are near a brick factory. The site T-5 which was assumed to be affected by the mine spoil Sherüj showed only a slightly elevated soil Cd concentration. The apparent transfer factors for Pb and Cd are much higher than literature values and are negatively correlated with soil and plant N and P supply (results not shown) propably due to trace element dilution in the biomass.

4. Conclusions

This work demonstrates for the first time a heavy metal pollution in Kirgisstan and provides evidence that Cd and Pb emissions from coal burning brick factories may be higher than sofar assumed.

Table 1: Research field locations

Field code	Location	Comment
T-2	Boo-Terek	Near main road
T-3	Ak-Döbö	Far from main roads
T-4	Keng-Aral	Near brick factory
T-5	Kara-Oj	Near mine spoil
N-2	Terek	Higher plant Na
N-3	Kara-Suu	Near main road
N-5	Ichke-Suu	Near brick factory

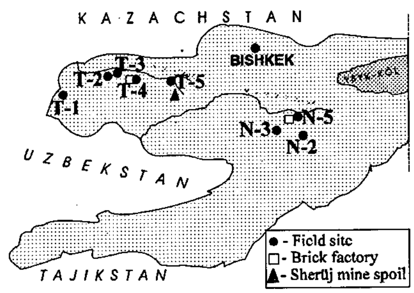

Figure 1: Project region Kirgisstan

Table 2: Trace element concentration[1)] in topsoils and apparent transfer factors to wheat

Site	Concentration in topsoil, $mg \cdot kg^{-1}$			Apparent transfer factors[2)]		
	Zn	Pb	Cd	Zn	Pb	Cd
T-2	90	1.4	0.12	0.12	0.08	0.35
T-3	106	1.1	0.08	0.14	0.09	0.38
T-4	65	3.9	0.78	0.14	0.21	1.19
T-5	71	1.6	0.17	0.14	0.08	0.24
N-2	54	1.6	0.15	0.28	0.09	1.07
N-3	53	5.4	0.76	0.27	0.08	0.89
N-5	69	7.5	0.86	0.34	0.13	2.28

1) Aqua regia extract

2) Apparent transfer factor = plant concentration/soil concentration

5. References

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EFFECTS OF LIMING ON CD SPECIES IN SOIL SOLUTION AND UPTAKE OF CD IN POTATOES

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1. Introduction

Liming has been suggested as a measure to reduce Cd uptake in arable crops, but earlier experiments has shown that liming might give the opposite effect. The reason for this discrepancy is that the large amount of Ca^{2+} applied will compete with Cd^{2+} for reactive sites in the soil. Therefore dolomite could be a "better" liming material since less Ca^{2+} is contained per alkalinizing equivalent. Many different soil extractants have been suggested for use in predicting the uptake of Cd, but the correlations has generally been low. In this study we investigated the hypothesis that; (i) dolomite is a "better" liming agent than calcite (ii) Cd speciation of the soil solution predicts the Cd uptake in the crop better than conventional extractants.

2. Materials and Methods

In two liming experiments, potatoes (*Solanum tuberosum*; var. King Edward) were grown in four replicate plots. The treatments at site 1 (Nättraby) were unlimed and limed with calcite to 55%, 70% and 100% base saturation (BS), while at site 2 (Kövlinge) the treatments were unlimed, limed with calcite to 55% and 70% BS, and limed with dolomite to 55% and 70% BS. The soil texture was a silt loam at site 1 and a sandy loam at site 2. Surface soil (0-25 cm, A_p) and potato tubers were sampled from all plots at harvest time. Soil characteristics such as "total"-Cd (2M HNO_3) and Cd extracted with NH_4NO_3 (Am-Cd) were determined. Potatoes were digested and analysed for Cd. To obtain soil solution, deionized water was added to fresh soil samples until saturation was reached and then incubated at 12°C for 24 h prior to centrifugation at 1500g and 30 min. After membrane filtration (0.2µm), pH, EC, concentrations of major cations and anions (ion chromatography), Cd (GFAA) and dissolved organic carbon (DOC) were determined. Activities of the various Cd species in the soil solution were calculated using the chemical model WHAM (Tipping, 1994).

3. Results and Discussion

At site 1 liming significantly affected Cd^{2+}, $CdCl^{+}$, $CdSO_4^{0}$, org-Cd^{2+} and pH in the soil solution as well as the Am-Cd. A significant effect on Am-Cd was also observed at site 2, but in the soil solution only Cd^{2+} and pH were significantly affected (Tab. 1). The activities of Cd species in the soil solution were low at both sites compared to other investigations (McLaughlin et al., 1997). The concentration of Cd in potatoes from site 1 was significant higher (ten times) than in tubers from site 2. The small, but significant (P=0.00) higher solution activity of Cd species at site 1 are not a likely explanation for this.

Tab. 1. Activities of Cd species, Ca^{2+}, pH and DOC in soil solution from two liming experiments. Concentrations of Am-Cd, HNO_3-Cd and potato Cd from the same experiments. Treatments; A) unlimed; B) calcite 55%; C) 70%; D) 100%; E) dolomite 55%; F) 70% BS

Site/ Treatm.	Cd^{2+} nM	$CdCl^+$ nM	$CdSO_4^0$ nM	Org-Cd^{2+}	Ca^{2+} mM	pH	Am-Cd µg/kg	HNO_3-Cd µg/kg	Cd-pot µg/kgDW
Site 1 (Nättraby)									
A	1.7^a	0.09^a	0.21^a	0.6^{ab}	1.8^a	5.6^a	19^a	140^a	380^a
B	1.3^{ab}	0.08^a	0.18^a	0.7^{ab}	1.6^a	5.7^{ab}	19^a	140^a	380^a
C	1.1^{ab}	0.06^{ab}	0.19^a	0.9^a	1.3^a	5.9^b	20^a	150^a	350^a
D	0.3^b	0.02^b	0.06^b	0.4^b	2.0^a	6.9^c	6^b	140^a	310^a
Site 2 (Kövlinge)									
A	0.7^a	0.05^a	0.19^a	0.6^a	0.7^a	6.0^a	8^a	100^a	33^a
B	0.5^b	0.03^a	0.13^a	0.7^a	1.0^a	6.6^b	4^b	100^a	38^a
C	0.6^{ab}	0.04^a	0.19^a	0.7^a	1.0^a	6.7^b	4^b	100^a	38^a
E	0.6^{ab}	0.04^a	0.20^a	0.7^a	0.8^a	6.5^b	6^a	100^a	35^a
F	0.5^b	0.03^a	0.15^a	0.7^a	0.7^a	6.6^b	5^b	110^a	42^a

The content of Cd in potatoes from site 1 was significantly related to Cd^{2+}, $CdCl^+$, $CdSO_4^0$ (positively) and pH (negatively) in the soil solution, as well as to Am-Cd (positively). Cadmium in potatoes from site 2 was only significantly related to pH (positively) in the soil solution (Tab. 2). The correlations between Cd in potatoes and Cd^{2+}, $CdCl^+$, $CdSO_4^0$, org-Cd^{2+} Ca^{2+}, and pH in the soil solution and Am-Cd were also tested in a stepvise multiple regression. At site 1 only $CdCl^+$ was included in the model (100 R^2=33), and at site 2 only pH was included (100 R^2=27). Site 1, Cd_{pot} = 300 + 8.6E+11*$CdCl^+$; Site 2, Cd_{pot} = -31 + 11*pH.

Tab. 2. Percentage of the variation (100 R^2) accounted for in simple linear regression relating potato Cd concentrations to the activity of Cd species, pH in the soil solution and Am-Cd

Site	Cd^{2+}	$CdCl^+$	$CdSO_4^0$	Org-Cd^{2+}	Ca^{2+}	pH	Am-Cd
1. Nättraby	*26(pos)	*38(pos)	*34(pos)	n.s.	n.s.	*31(neg)	*33(pos)
2. Kövlinge	n.s.	n.s.	n.s.	n.s.	n.s.	**39(pos)	n.s.

n.s., not significant; * $P<0.05$; ** $P<0.01$

4. Conclusions

Liming decreased the tuber Cd content at site 1 but increased it at site 2. The mechanism behind the adverse effect of liming needs to be further studied. No significant difference was found between the effects of dolomite and calcite. At Site 2 the speciation of the soil solution gave a better prediction of the Cd uptake in potatoes than Am-Cd , but at site 1 the Am-Cd was as good a predictor as the parameters in the soil solution.

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BIOAVAILABILITY OF HEAVY METALS IN SOILS IN THE VICINITY OF METAL MINING AREA, SOUTH KOREA

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1. Introduction

Heavy metals derived from mining area are dispersed and accumulated in soils, and may influence crop plants. Paddy rice is grown on the soils nearby a mining area including Duckum Au mine, South Korea. In order to determine the availability of heavy metals in soils, the tailings and soils were sampled and analyzed by sequential extraction analysis. Especially, the paddy rice was also sampled and analyzed in Duckum mine area, and the speciation of heavy metals that moved from soils to crops were compared with the soil pH and heavy metal concentrations in crop plants.

2. Materials and Methods

1) soil pH : 5g of air-dried soils (< 10mesh) were weighed into centrifuge tubes and 25ml ofdeionized water were added. The solutions were stirred intermittently for 1 hour and left for 1/2 hour. The pH was measured in the supernatant solution.
2) Acid digestion: For the aqua regia method, HNO_3 (1 ml) and HCl (3 ml) were added to the tailings and paddy soil (0.25 g) and heated at 70°C for 1 hour. After that, deionized water (6 ml) was added and analyzed by AAS (Atomic Absorption Spectrometry). Heavy metal concentrations in paddy rice were also analyzed by AAS after the removal of organic matter and these digestion procedures.
3) Sequential extraction: The 6-step extraction method suggested by FÖRSTNER (1986) was used. Step 1 the exchangeable fraction, step 2 carbonic fraction, step 3 corresponds to Mn oxides fraction, step 4 Fe oxides fraction, step 5 sulfidic/ organic fraction, and step 6 residual fraction (Figure 1).

3. Results and Discussion

The pH and heavy metal concentrations in tailings from the metal mining area are shown in Table 1. Lead concentrations in most tailings are higher than permissible level of soil in Korea and decrease in order of Imchun > Goobong > Sujum > Daduck > Dalsung > Ilkwang > Duckum mine areas. When the soil pH are considered, the availability of Pb is enhanced in soil with decreasing of soil pH. It means that the bioavailability of heavy metals including Cd, Cu, Pb and Zn may be discussed with the result of soil pH and chemical speciation in soils. In order to investigate this availability of heavy metals in tailing soils, heavy metal concentrations of soils and plants in the metal mining area are discussed.

Table 1 Soil pH and heavy metal concentrations in tailings from the metal mining area, South Korea (ug/g)

Mine	Ilkwang 1	Ilkwang 2	Imchun 1	Imchun 2	Daduck 1	Daduck 2
Soil pH	2.65	3.67	1.86	1.91	2.67	4.84
Zn	284	1520	16800	13600	1080	40000
Pb	1826	734	9960	9780	3354	3886
Cu	400	3240	300	220	80	1280
Cd	0	8	54	46	4	148
Fe	60480	44840	224400	207000	60320	70000
Mn	138	374	6	5	126	14840

Mine	Dalsung	Sujum	Sangdong	Gahak	Goobong	Sambo	Duckum
Soil pH	3.56	3.46	8.26	8.23	8.02	6.61	2.38
Zn	1160	14800	880	4360	4840	4400	840
Pb	2450	4774	166	1050	5822	538	1560
Cu	10000	160	560	560	400	40	124
Cd	8	44	16	28	136	4	8
Fe	88400	47440	82440	38760	28720	10800	-
Mn	278	7600	4200	1182	322	2240	-

Step 1 (EXCHANGEABLE FRACTION) Reagents NH4OAc, 1.0M, pH 7	Step 2 (Carbonatic Fraction) Reagents NaOAc, 1.0M. pH 5 with HOAc.	Step 3 ***(EASILY REDUCIBLE FRACTION)*** Reagents 0.1M NH2OH HCl with 0.01M HNO3
Step 4 (Moderate Reducible Fraction) Reagents 0.2 M Oxalate buffer, pH 3.	Step 5 (Sulfidic/Organic Fraction) Reagents 30% H2O2 with 0.02M HNO3, pH2. 1M NH4OAc, in 6% HNO3.	Step 6 (Residual Fraction) Reagents HNO3 and HCl

Figure 1: Sequential extraction procedure modified from FÖRSTNER (1986)

4. Conclusions

1) The pH of tailing from Imchun mine ranged from 1.86 to 1.91. A large amount of heavy metals were extracted by deionised water from this tailing compared with other tailings of higher pH.
2) From the result of sequential extraction analysis, it was found that most of heavy metals in tailings from mining area existed as sulfies/organic matter form or residual fractions. However, a number of tailings comprised significant portions of exchangeable forms in the range of 20 to 63 percent.

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MOBILITY AND AVAILABILITY OF METALS TO PLANTS

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The availability of metals to plants is their major characteristics in all the biogeochemical, soil, harvest and other investigations. It is closely connected with their mobility, especially solubility in a solid phase, presence in watersoluble ionic forms and especially in gas forms. We have allocated three models of a root nutrition of plants: litho -, hydro- and atmobiogeochemical (fig.) depending on a presence of a chemical element (CE) in the solid, liquid or gas phases. Their main difference between is sharply various intensity of CE absorption by the nonbarrier bioobjects of plants. As it is seen from data in table. 1, relations of average values of the plant-soil (PSC), plant-water (PWC) and plant-gas, rooted (PGC_r) coefficients differ from each other as 1:3000:300000. The second

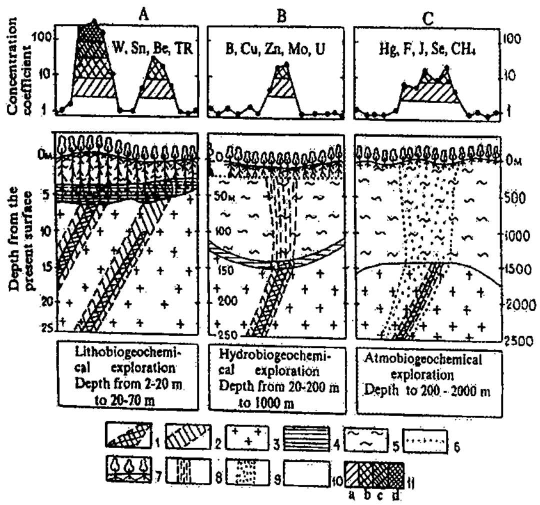

Fig. Main models of the biogeochemical exploration.
A – lithobiogeochemical, B – hydrobiogeochemical, C – atmobiogeochemical. 1 – ore bodies; 2 – primary haloes; 3 – native rocks; 4 – eluvium; 5 – allochtonous covers; 6 – water-bearing horizon; 7 – plants; 8 – ore hydrogeochemical haloes; 9 – ore atmobiogeochemical haloes; 10 – background contents of the indicator elements in profiles; 11 – anomal concentrations of various intensities: a – weak (3-10 backgrounds), b – middle (10-30 backgrounds), c – intensive (30-100 backgrounds), d – very intensive (>100 backgrounds).

Plant-water (PWC)	1000-10000	3000	
Plant-gas (root) (PGC_r)	$1\cdot10^5$-$1\cdot10^6$	$3\cdot10^5$	

difference of these models is a various site of nutritive horizons (NH). It is close to maximum root depths of investigated plant species (0,5-5m) in a lithobiogeochemical model. NH is usually situated on top of water-bearing horizon or in its capillary edging at depth 2-20 m, reaching 50-200 m in a hydrobiogeochemical model. The determinations of PWC are possible only in artificial water cultures. Primary sources of CE can be at depths, measured by tens and hundreds meters, which can be considered as NH in a atmobiogeochemical model. Plants give information about CE in the solid, liquid or gas phase, being at different depths, depending on model of a root nutrition. Determination of the root nutrition model of the investigated CE in concrete conditions is the first problem of interpretation of the CE observed contents in plants. The absorption of CE by plants most frequently occurs with the help of the simplest lithobiogeochemical model. The hydrobiogeochemical model is characteristic only for elements intensive water migrants at presence of free underground waters in the root-inhabited zone. In fact the most characteristic feature is the complex lithohydrobiogeochemical model because of CE sorption from waters by clay fractions of soils. The atmobiogeochemical model is possible only for elements gas migrants. It is rather peculiar for mercury. The majority of mercury biogeochemical anomalies is caused by maximum intensity of vaporous mercury absorption by roots of plants. The absorption of carbonic acid

CO_2 by shoots of plants takes place with average value of $PGC_{sh} \sim 3 \cdot 10^5$ by atmobiogeochemical model. The latter is established by special studies.

Table 2. Values of PSC for mineral forms of F, Zn, Cu, Hg, Pb of various size, for ash of the nonbarrier bioobjects of plants

Element	Bioobjects	30-300 / 100	3-30 / 10	0,3-3,0 / 1,0	0,3-0,03 / 0,1	0,03-0,003 / 0,01	0,0003-0,003 / 0,001
F	1,2	Villiaumite, fine-grained (<0,1mm) cryolith	oarse-grained (>0,3mm) cryolith fine-grained (<0,1 mm) fluorite	Coarse-grained fluorite, NM-forms	-	-	-
Zn	3,4,5	Sulphates, fine-grained sphalerite	Coarse-grained sphalerite, NM-forms	Fe-forms	-	-	-
Cu	1,2	Water-soluble forms	Chalkopyrite, tetrahedrite, covelline, NM-forms in pH of soils < 5,0, fine-grained malachite	NM- and Fe-forms in pH of soils > 6,0, middle (0,1-0,3mm) malachite	Fe-forms, coarse-grained (>0,3mm) malachite	-	-
Hg	1,2	Dispersed (<0,01mm) native mercury	Fine-grained (<0,1mm) native mercury	NM-forms	Dispersed (<0,01 mm) cinnabar	Fine-grained (<0,1mm) cinnabar	Coarse-grained (>0,1mm) cinnabar
Pb	1-5	-	Anglesite, cerussite, NM-forms	Wulfenite NM- and Fe-forms	Fine-grained (<0,1mm) galena	Middle-grained (0,1-0,3mm) galena	Coarse-grained (>0,3-1mm) galena

Notes: 1-5 – the corresponding nonbarrier bioobjects: 1-2 - bark of *Betula verrucosa*, *B. platyphylla*; 3-5 – outer layers of bark: 3 – *Pinus silvestris*, 4-5 – *Larix dahurica*, *L. sibirica*.

NM-forms – non-mineral forms.

Fe-forms – ferrous forms.

Data for mercury – without ~ 10-times recovery for its loss in ashing of plants.

When influence of free waters and gas forms is absent, the intensity of CE absorption by the nonbarrier bioobjects of plants is determined in the lithibiogeochemical model by two main factors: 1) the solubility; 2) size of mineral grains. A fragment of these factors studying results is shown in Table. 2.

The analysis of models in fig. 1 and data in table 2 show, that the intensity of absorption by plants of the various CE forms depends on energy of their connection in external environment. Quantitative estimations of this connection with energy of crystal lattices of minerals are possible.

It is necessary to note, that the requirement of CE for plants does not affect intensity of absorption. Answer-back reaction at a lack of CE is physiological, instead of absorption intensity increase.

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CONCENTRATIONS OF TRACE METALS IN CORN GROWN IN ARGENTINA

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1. Introduction

The anthopogenic accumulation of trace metals in crops can affect the quality of foods used for human consumption. The concentration of those metals in plant tissues comes, but not exclusively, from soils enriched with bioavailable trace metals. Car exhaust or atmospheric coontaminants can also affect plant metal concentrations. We have recently studied the concentration of seven trace elements (Cd, Co, Cr, Cu, Pb, Ni and Zn) in Mollisols of the eastern area of the Argentinean Pampas (Lavado et al, 1998). The concentrations of all studied elements in the agricultural soils of the area are around the average world concentrations and below the maximum tolerable thresholds (Frink, 1996).

However, the concentration of trace elements in crops in this area is still unknown. The area is devoted to cash crops e.g. corn. Our objective was to determine the trace metal concentration in corn grown in the Argentinean Pampas area.

2. Materials and Methods

An average of three composite samples of leaves and grains of mature corn were taken in six places (two experimental sites and four farms) in the northeast of Buenos Aires province. We sampled the hybrid Nidera AX-845 in three sites, the hybrid Cargill 845 in two sites and the hybrid Asgrow RX 405 W in one site. in March 1996 and 1997. Plant samples were dried at 70* C, grounded, digested by a mixture of hot acids (HNO_3-HCl_4H-H_2O_4S) and analyzed for B, Cd, Co, Cr, Cu, Mn, Mo, Pb, Ni and Zn. by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES).

3. Results and Discussion

Concentrations of the metals studied are summarized in Table 1. Comparing the obtained data with the information provided by different authors and regulations (Pais and Benton Jones, 1997), the concentrations of Cd, Co, Cu, Mn and Pb were within the normal range of values. The concentration of B and Zn belong to the same group, but showed some values in the lower end of the normal range. This could be due to the general deficiency of B and Zn in Pampean soils (Lavado and Porcelli, 1999). On the other hand, some concentrations of Cr, Ni and Mo are above the average but not excessive. For these elements there was a high variability in concentrations.

Corn trace metal concentrations are in accordance with the low content of trace metals found earlier in soils of the area (Lavado et al, 1998). Besides, the absorption of trace metals varies within and between species. Florijn and van Beusichem (1993) while studying cadmium in corn, found that concentration variability was independent of the root media but it had a genetic basis. This could be another factor to justify differences among corn cultivars, As usually indicated, the grains have lower concentration than leaves, for most elements.

Table 1: Trace metals concentration in leaves and grains of corn

	B	*Cd*	*Co*	*Cr*	*Cu*	*Mn*	*Mo*	*Ni*	*Pb*	*Zn*
					Leaves					
MINIM UM	2.94	0.05	< 0.5	< 0.5	2.0	28.67	1.58	0.45	0.48	27.43
Maximum	18.00	0.50	<0.5	3.90	11.99	61.00	2.00	2:0	5.00	46.87
Mean	9.09	0.21	--	--	6.78	42.35	1.71	1.31	0.71	36.56
					Grains					
Minimum	1.61	0.05	< 0.5	< 0.5	2.00	8.00	1.47	0.5	0.71	20.85
Maximum	5.67	0.50	< 0.5	1.16	8.8	12.50	2.00	2.00	5.00	30.5
Mean	3.43	0.20	--	--	4.18	8.5	1.53	1.18	2.71	23.83

4. Conclusions

The present study add new information to the general idea that the Pampean region is an uncontaminated area for trace elements.

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CADMIUM UPTAKE BY MAIZE AS INFLUENCED BY SOIL PH AND CADMIUM CONTENT

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1. Introduction

Cadmium has no essential biological function, and is highly toxic to plants and animals (Adriano, 1986). Food is the main route by which Cd enters the body. Availability of cadmium for plants is influenced by various soil factors, among them soil pH is of special importance (Alloway, 1995). The effect of increasing Cd concentration and different soil pH on the Cd uptake and biomass production of maize was studied in pot experiments.

2. Materials and Methods

Table 1. Some characteristic of the experimental soils.

Soil type	Location	Label	Cd mg/kg soil total	Cd mg/kg soil Lakanen-Erviö m	pH_{KCl}	Humus	Clay %	Silt
Eutric cambisol	Keszthely	KE	0.180	0.058	6.80	2.11	18.8	23.4
Gleyic luvisol	Szentgyörgyvölgy	SN	0.325	0.086	4.28	1.52	17.3	36.9

1kg soil was filled in plastic pots. The pots were kept at constant soil moisture. 4 Cd treatments used in the experiments were (Cd mg kg $soil^{-1}$) control=0; Cd_1=1; Cd_5=5; Cd_{10}=10. Cadmium was applied as cadmium sulfate in 4 replicates. Maize (*Zea mays* L.), cv. Pioneer 3732 SC was grown for 35 days, five plants per pot. Fresh and dry weight of plants were determined after harvest. Digestion with cc.HNO_3 was made by using microwave heating. Cadmium content of plant samples was determined by using an atomic absorption spectrometry.

3. Results and Discussion

Experiment with KE soil : There were no significant differences in shoot fresh and dry matter weights as a result of Cd treatments (Table 2). Cd concentration in shoots following treatments with increasing Cd concentration proved to be statistically significant. Correlation was also found between Cd treatments applied and Cd concentration of maize shoots, which can be described by a linear function: $y=0.1495x+0.3585$, $r^2=0.7395$, n=16. Increasing loads of Cd decreased the fresh and dry weigth of roots. Cadmium concentration of roots increased as a result of Cd treatments, and it was higher in all cases than that of the shoots.

Experiment with SN soil : A significant decrease was detected in the fresh and dry weights of shoots of maize plants, following treatments with increasing Cd concentration.

The increase in Cd loads on soil was found to be responsible for a considerable increase in the Cd concentration of shoots, which was by 29-64-125 times higher as a consequence of Cd_1-Cd_5-Cd_{10} treatments. Respectively the Cd concentration of plants in certain treatments slightly exceeded the Cd concentration of soil. The correlation between Cd treatments and the Cd concentration of shoots can be described by the following linear function:

y=1.164x+0.8246, r^2=0.956, n=16. The cadmium uptake of plants was increasing in the order of the treatments. Increasing Cd levels generated a decrease in the fresh and dry weights of roots, while there was a significant increase in the Cd concentration of roots.

Table 2. Effect of Cd treatments on the biomass production and Cd concentration of maize

Cd	fresh weight g/pot		dry m. weight g/pot		Cd mg/kg d.w.		Cd uptake µg/pot	
treatments	root	shoot	root	shoot	root	shoot	root	Shoot
				Eutric cambisol (KE) soil				
control	33.74 a	25.47 a	3.51 a	3.72 a	1.37 a	0.001 a	4.78 a	0.004a
Cd_1	29.45ab	23.46 a	3.28 a	3.55 a	5.98 b	0.75 b	19.07 b	2.66 b
Cd_5	26.86 b	22.99 a	3.16 a	3.71 a	6.10bc	1.39 c	19.69 b	5.14 c
Cd_{10}	25.90 b	23.71 a	3.13 a	3.66 a	7.31 c	1.69 c	22.83 b	6.17 c
$LSD_{5\%}$	4.75	2.77	0.46	0.29	1.26	0.39	4.09	1.39
				Gleyic luvisol (SN) soil				
control	25.56 a	27.32 a	3.70 a	4.52 a	1.13 a	0.10 a	4.19 a	0.49 a
Cd_1	25.53 a	26.88 a	3.44ab	4.27ab	14.28 b	2.94 b	48.86 b	12.65 b
Cd_5	23.22 a	23.35 b	3.07 b	3.60 c	19.10 b	6.37 c	59.51 b	22.75 c
Cd_{10}	23.45 a	21.98 b	3.30ab	3.71bc	28.05 c	12.51 d	93.03 c	46.06 d
$LSD_{5\%}$	3.93	3.15	0.42	0.60	7.99	1.37	30.31	3.07

Comparison evaluation of results obtained with the two soils : The Cd concentration of plants grown on strongly acidic soil and the amounts of Cd taken up by them were considerably higher than with those grown on neutral KE soil. The amount of Cd taken up by maize on SN soil was by 4.7-4.4-7.5 times higher than on neutral KE soil. The Cd concentration of roots was several times higher (3-8 times) than that of shoots, in both soils. These results indicate that a considerable part of Cd was not translocated to the shoots but remained in the roots.

4. Conclusions

The biomass production of maize decreased as a result of Cd treatments, its rate showed differences depending on the soil. Cadmium concentrations in roots and shoots increased with Cd treatments. No phytotoxic symptoms were observed on the plants during the experiment. The Cd concentration of the roots of maize plants was higher in all the treatments on both experimental soils, than that of the shoots; by 4.3-8.0 times higher on neutral KE soil, while the difference was by 2.2-4.8 times higher on strongly acidic SN soil, which proves that only a part of Cd was translocated to the shoots. This difference between the Cd concentration of roots and shoots decreased by increasing Cd loads. The Cd concentration of plants grown on acidic soil was several times higher than of plants grown on neutral soil. In certain treatments shoots Cd concentrations were 3.9-7.4 times higher, while roots concentrations were 2.4-3.8 times higher in the acidic soil. The shoots and roots of plants grown on acidic soil indicated higher Cd concentrations in all the treatments than in the soil. It can be concluded that soil pH played a decisive role from the point of view of Cd uptake by maize plants.

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COPPER AND NICKEL ACCUMULATION IN *EMPETRUM NIGRUM*

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1. Introduction

Empetrum nigrum (crowberry) is one of the few understorey species which grows on sites highly contaminated by heavy metals in the vicinity of Cu-Ni smelteries in the Northern hemisphere. Tissue concentrations exceeding 1000 mg/kg of Cu and Ni have been reported (CHERTOV et. al, 1993; HELMISAARI et al., 1995; UHLIG et al., 1996) and therefore *E. nigrum* can be classified as a hyperaccumulator of these elements. However, high concentrations have been measured from field samples and results might be partly due to surface contamination. The aim of this research was to investigate the pattern of accumulation of Cu and Ni in *E. nigrum* under controlled conditions.

2. Materials and Methods

E. nigrum seedlings, propagated from cuttings, were sourced from an unpolluted area. The response of seedlings to Cu or Ni was investigated in a greenhouse experiment. Plants growing in quartz sand were treated over a six weeks period with 50 ml nutrient solution containing different levels of either Cu or Ni (0/1, 10, 22, 46 and 100 mg/l). Control solution contained 0 mg/l Ni but 1 mg/l Cu because plants do not necessarily need nickel for growth. Length growth, discolouration of leaves, and element composition of different plant parts were measured. After the experiment plants were divided in nine parts which were; 1) green leaves of current year's growth, 2) discoloured leaves of current year's growth, 3) green leaves of previous year's growth, 4) discoloured leaves of previous year's growth, 5) older green leaves, 6) older discoloured leaves, 7) stems of current year's growth, 8) stems of previous year's growth, 9) older stems. Samples were dried at 60°C, weighed and chemical composition was determined by ICP-spectrometer after dry ashing. For chemical analysis leaves of current and previous year's growth and stems of current and previous year's growth were combined.

3. Results and Discussion

In *E. nigrum* increasing concentrations of Cu or Ni in nutrient solutions caused 1) increasing discolouration of leaves, 2) increasing Cu and Ni concentrations in all investigated plant tissues, 3) decrease in plant height, 4) decrease in biomass. However, all the plants survived during the experiment. The biomass of discoloured leaves of the two newest year growths as a percentage of the total leaf biomass in these age classes was 48% for the highest Cu treatment and 59% for the highest Ni treatment. During the six weeks period *E. nigrum* accumulated over 2000 mg/kg of Cu or Ni in older stem tissue and metals seemed to accumulate with age, so that younger stems contained less metals than older stems (Fig 1). In general, green leaves contained lower Cu and Ni

concentrations than discoloured leaves of comparable age. The maximum growth reduction of seedlings treated with 100 mg/l Cu was 73% whereas it was 90% of seedlings treated with 100 mg/l Ni, compared to the growth of control seedlings. In general, Ni affected growth and discolouration of *E. nigrum* at lower concentrations than Cu.

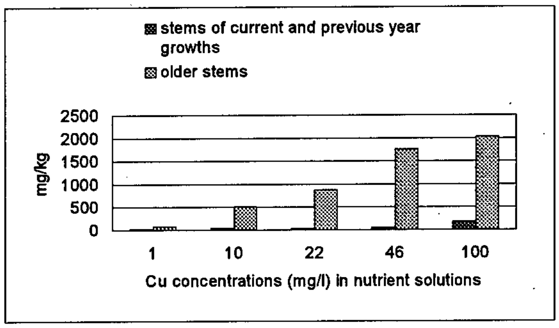

Fig. 1. Copper concentrations (mg/l) in stems of *E. nigrum* in response to Cu concentrations in nutrient solutions (8 plants in one bulk sample).

4. Conclusions

Pot experiments confirmed the results of field studies that 1) *E. nigrum* accumulates Cu and Ni over 1000 mg/kg and 2) the highest concentrations can be found in old stem tissue. This classifies *E. nigrum* as a hyperaccumulator of Cu and Ni, accumulating these metals selectively in the stem, especially in older tissue. *E. nigrum* seems to possess a constitutional heavy metal tolerance mechanism which is not based on genetical differentiation, because even seedlings collected from unpolluted areas tolerated high concentrations of Cu and Ni.

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UPTAKE OF CD^{2+} BY *SORGHUM BICOLOR*

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1. Introduction

Plant uptake is one of the major pathways by which toxic metal ions enter the food chain. On the other hand some plant populations can colonise contaminated environments, contributing for the development of phytoremediation (1-5).

Among different heavy metals, cadmium is of particular interest because it can accumulate in plants to levels that are toxic to humans and other animals but below toxic level to plants (6). Heavy metals may interfere with the biomass production and with the uptake of nutrients (7).

In this work the accumulation of Cd^{2+} in *Sorghum Bicolor*, in contact with a contaminated solution, is determined. The influence of Cd^{2+} on the growth and on the release of organic compounds by the plant as well as on the uptake of nutrients is also presented.

2. Experiments

The *Sorghum Bicolor* was grown in a non-contaminated / nutritive solution (solution I), in a temperature and luminosity controlled room. After one week the plant was immersed in a new solution (solution II), nutritive or not, with defined cadmium contamination levels (0, 0.01, 0.1, 1 ppm). A standard 0.1 M cadmium nitrate solution was used. After a certain contact time (from 5 to 20 days) the metal uptake by the plant is determined. A previous treatment using acid dissolution with microwave heating is performed in root and leaf experiments. In solution II the pH was decreased to 2 before the determination of the total metal concentration. Flame Atomic Absorption and/or Anodic Stripping Voltammetry were used as analytical tools to determine the total metal concentration.

The concentration of organic compounds with proton affinity released by the plant was determined by potentiometric acid/base titration.

3. Results and discussion

Cadmium uptake and plant development

The influence of cadmium contamination was evaluated in terms of biomass production, leaf feature (colour, height and thickness), nutrients and cadmium uptake.

The results show that the presence of Cd in small amounts increases the biomass production of *Sorghum Bicolor* as well as the plant feature in terms of colour, height and thickness of leaves. The higher biomass was found in the presence of cadmium 0.1 ppm, either in nutritive or non-nutritive solution. For 1 ppm the plant died after 5 days in non-nutritive solution, but not in nutritive solution, where the leaves were even thicker than in the absence of contamination.

From Table 1 it can be seen that cadmium uptake is always greater in non-nutritive solution. In both solutions, nutritive or not, the uptake of cadmium increases with the increase of the contamination level. The plant survives for higher levels of contamination in the presence of nutrients. From table 2 it can be found that the uptake of nutrients, per unit weight of biomass, is in general not significantly affected by cadmium contamination. Although the uptake of cadmium increases with the

contamination level, the adsorbed value per unit weight of biomass is similar after 5 or 20 days of solution / plant contact, for each level of Cd contamination.

Table 2 – Nutrients and cadmium uptake (mg of nutrients or Cd per g of biomass) after 5 and 10 days of plant / solution contact (results A and B respectively). The Cd contamination levels of 0, 0.1 and 1 ppm are represented, respectively, by numbers 0, 1 or 2 after A and B.

Table 1 – Cd uptake after d days of contact between solution and plant.

solution type / d days \ Cd contamination level	0.01 ppm	0.1 ppm	1 ppm
water / 5 days	94%	80%	38%
Hoagland / 5 days	—	28%	16%
Hoagland / 20 days	—	96%	70%

	Mg	Ca	K	Mn	Fe	Cd	Biomass g
A0	4	-	34	0	1.8	0.00	0.35
A1	3	-	31	0	0.6	0.05	0.6
A2	4	-	21	0	-	0.4	0.4
B0	3	21	39	1.6	0.5	0.00	1.6
B1	6	17	44	0.6	0.8	0.04	2.6
B2	6	21	43	1.2	0.9	0.4	1.7

Exudate parameters

The compounds with proton affinity, released by the plant after 5 days in a non-contaminated / non-nutritive solution, have concentration and stability constant values of $8x10^{-4}$ M and 4.6 (in log units), respectively.

In the presence of 0.1 ppm Cd contamination the protonation constant remains constant but the concentration is about twice the value found in non-contaminated solution. The increase of concentration may be due to the increase of root-mass production and not to any kind of external defence mechanism.

The values of DOC are within the experimental error of the determination ($\leq$ 5 ppm) for both experiments, in contaminated and non-contaminated solution.

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EFFECTS OF PH AND LIMING ON TRACE ELEMENT CONTENTS IN SPRING WHEAT AND POTATOES

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1. Introduction

A decrease in pH of arable soils due to acid deposition and other acidifying processes connected with agriculture (fertilisation, harvest of biomass, etc.) in combination with insufficient liming may lead to an increased plant uptake of trace elements, such as Cd, Zn, Ni and Cu. At the same time elements like Se will become less available. Basic foodstuffs are important sources of essential as well as toxic trace elements; i.e. cereals, potatoes and vegetables account for more than 70% of the total Swedish dietary intake of Cd, whereas cereals account for about 10% of the Se intake. The present study have been focused on the effects of soil pH on trace element levels in crops from a food quality point of view (Oskarsson et al., 1996; Thuvander & Oskarsson, 1998).

2. Materials and Methods

Seven long-term field experiments located in various agricultural regions in Sweden were sampled. Each experiment included four lime treatments (and four replicates): unlimed (~50 % BS) and treated with enough lime ($CaCO_3$) to reach 55, 70 and 100% base saturation (BS), respectively. Potato tubers (4 trials) and spring wheat grain (3 trials) were analysed for Ni, Mn, Cu, Cd, Zn, Al and Se during two subsequent years (on 5 of the sites) as described earlier (Öborn et al., 1995; Öborn & Jansson, 1998).

3. Results

Spring wheat grain. *Cadmium* levels varied between sites, averaging 99, 78 and 45 µg Cd kg^{-1} dw (Fig. 1a). Liming increased wheat Cd significantly (by 31 %) at one site, but tended to decrease grain Cd levels at the two other sites by about 25%. In all experiments the *Mn* (Fig. 1b), *Ni* and *Zn* levels decreased significantly with increasing lime rate (increasing pH), in average 29, 38 and 18%. The Mn, Ni and Zn conc. were in the range 24-43, 0.12-0.69 and 22-35 mg kg^{-1} dw, respectively. The wheat *Cu* level decreased by 22% (4.1 to 3.2 mg kg^{-1} dw) at one site, but no significant response was obtained in the others. The wheat *Al* conc. was 1.3-1.7 mg kg^{-1} dw and not significantly influenced by liming. *Selenium* levels tended to increase in response to liming, but the differences were not significant. There was a large difference in Se levels between sites, the averages being 4, 6 and 15 µg Se kg^{-1} dw, respectively.

Potato tuber. The potato *Cd* conc. varied widely between the sites, averaging 359, 70, 41 and 32 µg Cd kg^{-1} dw (Fig. 1c). Liming decreased the Cd conc. by about 20% at two sites, but it increased by 40% at the two other. *Mn* (Fig. 1d), *Ni* and *Zn* conc. in potatoes decreased significantly in response to liming, in average to 45, 20, 13% compared with the no-lime treatment. Potato Mn levels were significantly lower at one site, where also symptoms of Mn deficiency were observed at the highest lime rate. The average Zn conc. differed greatly between sites (11-21 mg kg^{-1} dw). The potato *Cu* conc. was significantly lower at the highest lime rate in one experiment, whereas in the other experiments no significant effect could be found. The *Al* and *Se* conc. in potatoes were not significantly affected by liming. The Al conc. was 0.4-0.9 mg kg^{-1} dw and the Se level was very low (1-3 µg Se kg^{-1} dw).

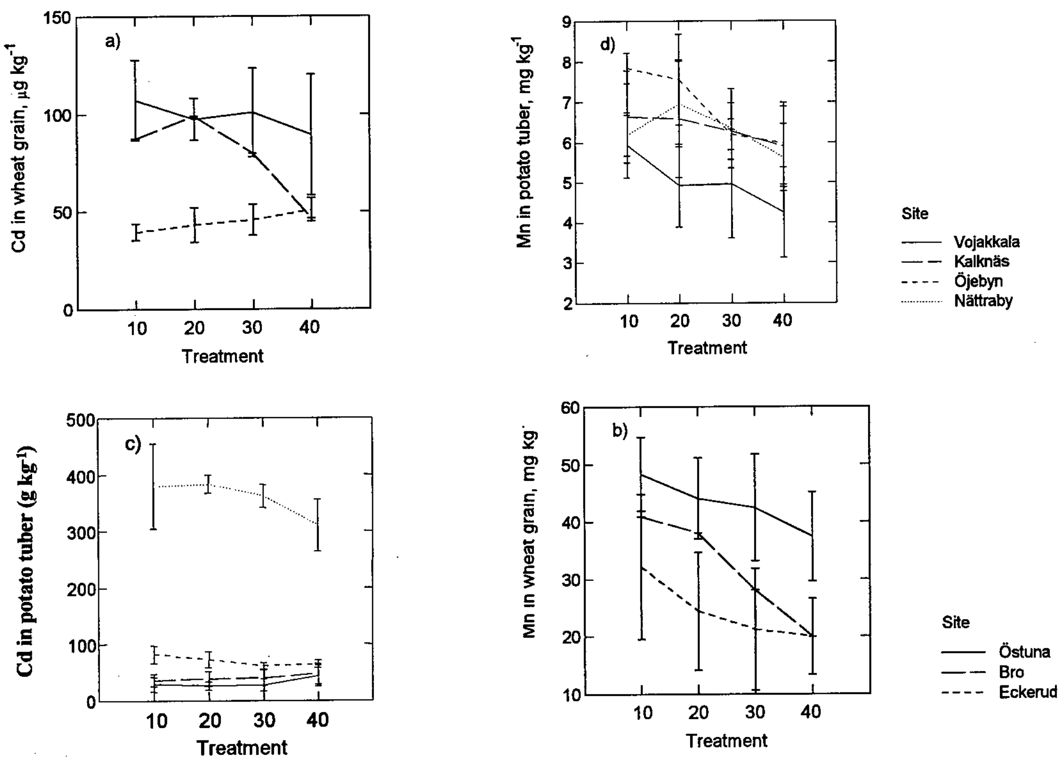

Figure 1. Effects of liming on spring wheat grain Cd (a) and Mn (b) conc. in three trials and on potato tuber Cd (c) and Mn (d) conc. in four experiments (per dry weight). The means and standard errors are shown in the graphs. The treatments are 10=unlimed, 20=limed to 55 % base saturation (BS), 30=limed to 70% BS and 40=limed to 100% BS.

4. Conclusions

Levels of trace elements in potato tuber and spring wheat grain showed very similar responses to liming, i.e. to increases in pH. The crop levels of Ni, Mn and Zn decreased with increasing liming rates, whereas the effects on Cd and Cu were inconsistent, and Se and Al were not significantly affected. For most elements the differences in crop conc. were larger between sites than between liming treatments. The effects of parent material, soil properties and other site specific factors have to be further investigated.

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EFFECTS OF CHEMICAL FORM OF NITROGEN SOURCE ON HEAVY METAL UPTAKE BY HIGHER PLANTS

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1. Introduction

The uptake of heavy metals by higher plants from soil depends on a variety of factors, such as soil pH, soil type, and organic matter content (BINGHAM et al. 1979, 1980; MAHLER et al. 1978; MITCHELL et al. 1978). Some of these factors can be easily changed by the addition of various materials to the soil, such as inorganic fertilizers and organic manure and sludges. Nitrogen, one of the most important elements in plant nutrition, is supplied mainly by inorganic fertilizers. It is classified into 2 main groups, nitrate nitrogen (NO_3) and ammonium nitrogen (NH_4). Because nitrate increases soil pH and ammonium decreases it during cultivation, heavy metal uptake by plants could be affected by the application of nitrogen compounds.

This study investigated the influence of both the nitrogen form and its counter ion on heavy metal uptake by 3 plants in 2 types of soil.

2. Materials and Methods

Plants: Rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.), and spinach (*Spinacia oleracea* L.) were used. **Soil:** Gray Lowland soil, with a weak buffer action, and Andisol, with a strong buffer action for soil pH change, were used at 0.5 kg and 0.3 kg per 1 L pot, respectively. **Nitrogen compounds:** We added 150 mg N kg^{-1} to each pot, using $NaNO_3$ as a nitrate compound; NH_4NO_3 as a nitrate and ammonium compound; $(NH_4)_2SO_4$, NH_4Cl, and $NH_4H_2PO_4$ as ammonium compounds; and $(NH_2)_2CO$ as an organic compound, because of its proton loading potential. **Heavy metal treatment:** This consisted of 2 mg Cd, 10 mg Cu, and 120 mg Zn per kg of soil, as sulfates. P and K application: K_2SO_4 and $Ca(H_2PO_4)_2$ supplied 150 mg K_2O and 150 mg P_2O_5 per kg of soil. **Cultivation:** Fifteen seeds of each plant were sown in each pot. After 1 week, plants were thinned to leave 10 plants in each pot. Plants were harvested about a month after sowing. **Soil solution collection:** Soil solution was collected from the spinach pots in a porous cup. **Soil pH measurement:** After harvest, soil was collected from each pot, dried, and then mixed with 25 mL distilled-deionized water per 10 g dry soil. The pH of the suspension was measured by pH electrode. **Heavy metal determination:** Plants were dried at 75°C for 48 h, then digested with an HNO_3–$HClO_4$ mixture (5:3), and diluted to a volume of 25 or 50 mL with distilled-deionized water. The concentrations of Cd, Cu, and Zn in the digestion solutions and soil solutions were determined by using an atomic absorption spectrophotometer equipped with a carbon furnace.

3. Results and Discussion

In Gray Lowland soil, the soil pH after harvest was less than the initial soil pH and varied by nitrogen source: $NaNO_3$ gave the highest soil pH. In contrast, the final soil pH of the Andisol changed little in comparison with the initial pH (data not shown).

In Gray Lowland soil, Cd uptake by all 3 plants was highest with NH_4Cl application (Table 1). In contrast, Cu and Zn uptake differed among species (data not shown). In Andisol, nitrogen source did not affect heavy metal uptake (data not shown).

Table 1: Cd concentration in shoots of 3 plants grown in Gray Lowland soil (mean ± standard deviation; n = 3)

$\mu g\ g^{-1}$ dry weight	$NaNO_3$	NH_4NO_3	$(NH_4)_2SO_4$	NH_4Cl	$NH_4H_2PO_4$	$(NH_2)_2CO$
Rice	49.9 ± 3.0	16.9 ± 0.4	31.8 ± 6.9	57.7 ± 5.2	24.7 ± 3.6	15.9 ± 0.6
Barley	8.63 ± 0.93	10.0 ± 0.2	10.6 ± 0.7	11.5 ± 0.7	9.14 ± 0.02	7.01 ± 2.33
Spinach	19.0 ± 1.5	22.1 ± 3.3	28.6 ± 1.1	39.9 ± 1.1	24.8 ± 0.4	18.9 ± 5.7

Table 2 shows Cd concentrations in soil solutions collected from the Gray Lowland soil. NH_4Cl application gave by far the highest Cd concentration.

Table 2: Cd concentration in soil solutions collected from Gray Lowland soil after spinach harvest (mean ± standard deviation; n = 3)

$mg\ L^{-1}$	$NaNO_3$	NH_4NO_3	$(NH_4)_2SO_4$	NH_4Cl	$NH_4H_2PO_4$	$(NH_2)_2CO$
Cd conc.	0.292 ± 0.153	0.253 ± 0.088	0.285 ± 0.049	0.656 ± 0.008	0.123 ± 0.006	0.160 ± 0.156

Elevated soil Cl concentration increases Cd availability to field-grown crops (Li et al. 1994). This effect has also been found in greenhouse experiments using soils to which Cd was added as inorganic Cd salts (Bingham et al. 1983). Our results may therefore indicate that application of NH_4Cl to soils possessing a weak buffer action, such as Gray Lowland soil, increases Cd availability to plants not by decreasing soil pH but through the effect of Cl. Further experiments are now in progress to find the mechanisms of this effect of NH_4Cl.

4. Conclusions

Heavy metal uptake by 3 plants grown in Andisol was not affected by nitrogen source. In contrast, Cd uptake in Gray Lowland soil was greatly increased by NH_4Cl application.

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ZINC AND MANGANESE IN SOIL AND IN TOPS AND ROOTS OF TWO FORAGE LEGUMES GROWN IN A SOIL WITH FOUR BASE SATURATION LEVELS.

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1. Introduction

Soil acidity is related to legumes growth and nodulation. Liming changes nutrient availability in soil and, as a consequence, in the amount absorbed by plants. Zinc and manganese usually have their availability decreased as the soil base saturation (and pH) is increased.

The main objective of this work was to study zinc and manganese concentrations in soil and plant tops and roots in relation to variable soil base saturation.

2. Materials and Methods

Perennial soybean (*Neonotonia wightii* Lackley var. Tiranoo) and stylo (*Stylosanthes guianensis* Sw. cv. Bandeirante) were grown in a Dark Yellow Latosol limed to obtain four levels of soil base saturation (10; 30; 50 and 70%). Calcium and magnesium oxides rates (0; 1.2; 2.4 and 3.6 Mg ha^{-1} limestone equivalent) were applied to the soil. Macronutrients (except for N) and B, Cu, Mo and Zn (this one at 2kg ha^{-1}) were supplied.

Plants were grown in a greenhouse and were harvested twice in a completely randomized experiment, with five replications.

3. Results and Discussion

At the end of the soil incubation with liming material and before planting, soil pH (in $CaCl_2$ 0.01M) was 4.1, 4.4, 4.8 and 5.2 for the four liming rates.

Soil extractable (with HCl 0.1 M) Mn at the first and second harvests were 3.86 and 3.76 mg kg^{-1}, whereas for Zn they were 1.26 and 1.29, respectively.

Concentrations of the two micronutrients in plant tops and roots were highly correlated in both forage species. These plant nutrient concentrations decreased as the soil base saturation increased (Figures 1 and 2), and were negatively correlated to forage dry matter production. For perennial soybean at the second harvest. the correlation coefficient for dry matter production and micronutrient concentration was 0.92 for both Mn and Zn.

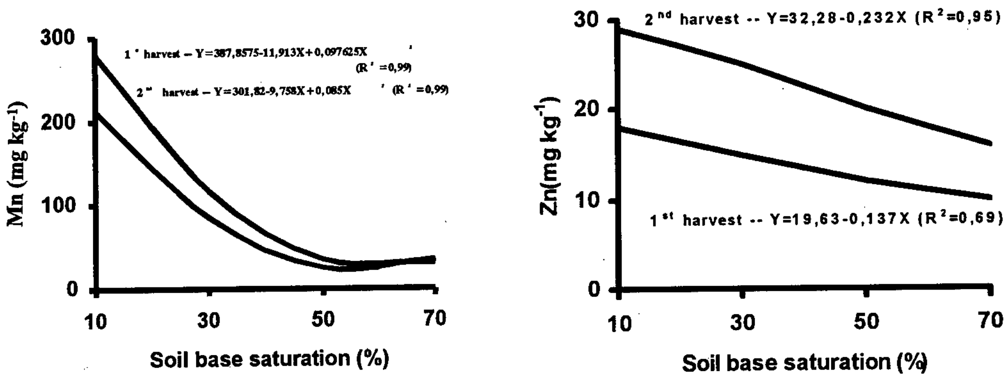

Figure 1 – Concentrations of Mn and Zn in perennial soybean tops related to percentage soil base saturation.

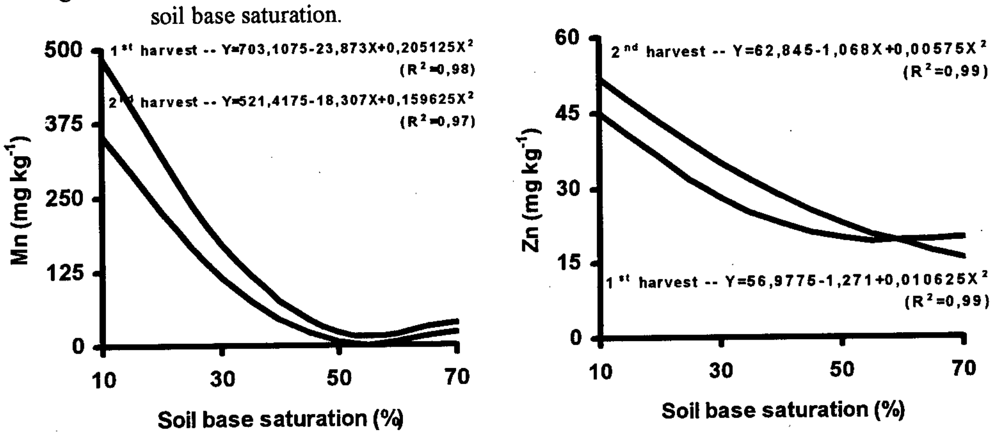

Figure 2 – Concentrations of Mn and Zn in stylo tops related to percentage soil base saturation.

At high soil base saturation (>50%) these micronutrients may be deficient for the two forage legumes. In that situation micronutrient concentrations of 20-30 mg kg^{-1} for Mn and 10-20 mg kg^{-1} for Zn in the top plant tissue were found in both species.

4. Conclusion

Soil extractable Mn and Zn (with KCl 0.1M) did not change with soil base saturation level.

Concentrations of Mn and Zn in plant top and root tissues of perennial soybean and stylo decreased with increasing soil base saturation. Zn may deficient at high soil base saturation level even with Zn applications to the soil.

CADMIUM UPTAKE AND ACCUMULATION CHARACTERISTICS IN A RANGE OF VEGETABLE CROPS.

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1.Introduction

Heavy metal contamination of agricultural soils poses a serious health risk as well as an environmental concern. The use of phosphatic fertilizers in particular contributes large quantities of cadmium to the soil (Alloway, 1995), which is readily taken up by some plant species and amplified through the food chain. Cadmium can be phytotoxic, although responses vary; resistance and accumulation have been observed (Punshon, et al 1997). This is of concern with regard to food chain transfer.

Wide-range vegetable crop screening cadmium resistance and accumulation was carried out as a pre-cursor to select in test crops for a series of soil amendment efficacy experiments. Information regarding the most popular vegetable crops grown in the UK on a per hectare basis (1995/1996) was used as a basis from which to select test. Currently available cultivars were selected and varietal differences were not considered in initial screening whose aim was establish comparative cadmium resistance and uptake between a range of vegetable crops.

2.Materials and Methods

Plants used in the screening experiments were obtained as seeds and raised in a controlled temperature glasshouse in John Innes No. 2 compost until the 4-leaf growth stage. Roots were carefully cleaned in distilled water, and seedlings were introduced to a hydroponic cultivation system. The plants were inserted through black, heavy gauge mulching plastic into 2 liter containers of aerated, full strength Hoagland's Solution (Hoagland and Arnon, 1941) (pH 5.8). This small-scale suspension hydroponic system was used (i.e. no submerged support matrix) to prevent interaction with heavy metals and nutrient solution was formulated with reagent grade chemicals. Treatments were 0, 0.01, 0.1, 1.0 and 5 mg L^{-1} cadmium, added as $2CdSO_4.5H_2O$. Solutions were changed every seven days. At least 30 replicate plants per treatment were used. Vegetables tested were as follows: wheat, barley, spinach, radish, lettuce, oil seed rape, Savoy cabbage, red cabbage, Chinese cabbage, cauliflower, calibrese, brussel sprouts, carrot and leek. Wet and dry biomass measurements were taken after one month, and material was separated in to leaves and roots and analyzed for Cd by AAS.

3.Results and Discussion

There was a strong relationship between growth and uptake for all crop seedlings tested. The overall trend in this respect suggests that a greater concentration of cadmium typically accumulated within root tissue on a $\mu g\ g^{-1}$ d.wt basis. Calabrese and cauliflower have the highest level of overall accumulation, although it appeared that there was comparatively little Cd accumulation in cauliflower until a threshold level of 1.0 mg Cd L^{-1} was reached. Red cabbage (cv. 'Red drumhead') accumulated the lowest concentration of Cd of all the plants tested in this study (Figure 1).

Table 1. Separation of test crops into uptake categories, showing toxic thresholds for cadmium in nutrient solution.

Crop Species	INDICATOR	ACCUMULATOR	EXCLUDER	Cd Threshold mg Cd L^{-1}
Wheat	✓			0.1
Barley	✓			0.1
Spinach			✓	>5.0
Radish	✓			>5.0
Oil Seed Rape	✓			0.1
Lettuce	✓			0.01
Savoy cabbage			✓	0.1
Red cabbage 'autoro'	✓			0.1
Red cabbage 'drumhead'			✓	0.01
Chinese cabbage		✓		0.1
Cauliflower		✓		0.1
Calabrese		✓		0.01
Brussel Sprouts	✓			0.1
Carrot	✓			0.1
Leek				0.1

4.Conclusions

From the data obtained in this study the vegetable crops can be *very broadly* grouped into uptake categories, depending on growth response and cadmium uptake characteristics (Table 1). The data indicates that the majority of crop species used could be used to indicate the presence of available Cd in the soil, and hence be used to assess the efficacy of soil amendments. This study suggests that when remediating Cd-contaminated soil, species selection should take into account the leaf and root accumulation characteristics; high leaf accumulation, observed in Chinese cabbage, cauliflower and calabrese would result in enhanced food chain biomagnification and should therefore be avoided.

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(a) *(b)*

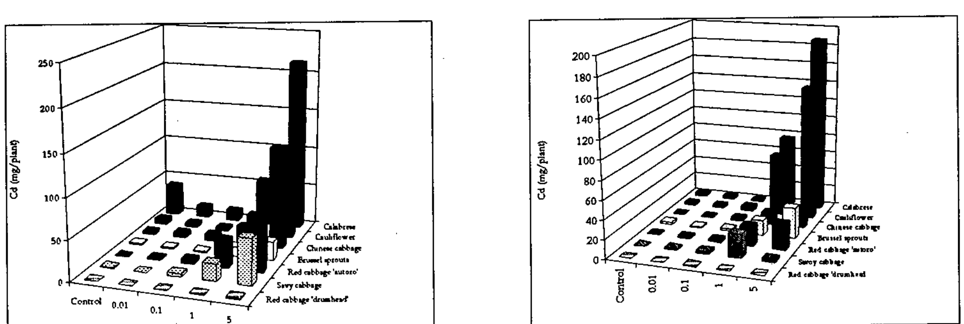

Figure 1. Cadmium uptake (mg plant^{-1}) in brassica leaves (a) and roots (b) after growth in solution for 28 days.

ACTINIDES BIOAVAILABILITY IN SOILS

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1. Introduction

Despite numerous data, knowledge on the long term availability of actinides in soils is still lacking (PRICE 1973, ADRIANO et al., 1986). The distribution ratio K_D is widely used for modelling their fate in soils. Among soil characteristics, we studied two parameters liable to influence the K_D values of neptunium and americium:

- the hydric conditions which in classical determination of K_D (liquid/solid ratio greater than unit) are far from the *in situ* state and,
- the concentration of some carboxylic acids in soil, liable to influence the sorption - desorption processes, CAWSE and COLLE (1989), ROUSSEL-DEBET (1996), TANAKA et al. (1997).

2. Materials and Methods

An acidic sandy soil and a calcareous soil, contaminated ten years ago with 18.5 $Bq.g^{-1}$ of ^{241}Am and 7.4 $Bq.g^{-1}$ of ^{237}Np were investigated. Extracting solutions were made either with osmosed water or with citric, oxalic, lactic or vanillic acids, 0.1 M to 0.001 M. These acids are exuded by roots or produced by microbial activity in the rhizosphere. Three methods were used to measure the radionuclide desorption: a) batch experiments - 10 g of dry soil + 50 ml of solution -, b) percolation - 50 g of soil slowly leached by 200 ml of solution - and c) centrifugation of soil initially wetted at field capacity with increasing accelerations - 1000 to 7500 *g* - ensuring a final moisture content of 10 %. Samples were mineralised in HNO_3. Neptunium was measured by low-level gamma Ge detection and ^{241}Am by alpha spectrometry, after radiochemical separation.

3. Results and Discussion

Table 1 shows how the moisture conditions of the soil affects the value of K_D: data obtained with realistic (unsaturated) conditions are lower than values measured for saturated conditions. Therefore, using classically (saturated) determined value would lead to an underestimate of the actinides mobility and subsequently of their transfer in terrestrial environment.

Table 1 : Effect of the hydric conditions on the distribution coefficient K_D ($Bq.g^{-1}$/ $Bq.ml^{-1}$)

	Soil	Unsaturated (centrifugation)	Percolation	Saturated (v/m =5)
^{241}Am	acidic	771 ± 19	988 ± 96	4260 ± 561
	calcareous	5740 ± 189	6295 ± 230	6333 ± 666
^{237}Np	acidic	24 ± 2	313 ± 120	142 ± 39

The addition of citric acid increases significantly the actinides desorption in the three kinds of experiment as reported, in Figure 1. So, the values of K_D determined with non - rhizospheric soil might be overestimated. Furthermore, measuring organic acids concentrations might be helpful for estimating seasonal variations of actinides migration and root transfer - which might be enhanced by labile organic matter -, and for modelling the long term behaviour of transuranics, in association with the organic matter cycle.

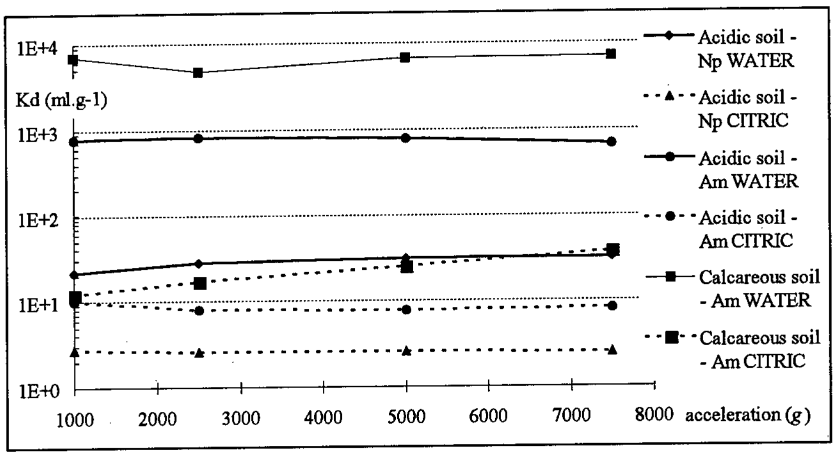

Figure 1 : Influence of citric acid 0,1 M on the distribution ratio K_D ($Bq.g^{-1}/ Bq.ml^{-1}$) determined by extracting the soil solution by centrifugation.

4. Conclusions

For better modelling of the dynamic availability of actinides in soil, more realistic hydric conditions and occurrence of carboxylic acids - i.e. biologically active root system - should be considered when measuring the distribution coefficient K_D.

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ACCUMULATION OF HEAVY METALS BY *SESBANIA* SPECIES

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1. Introduction

Environmental contamination has become a global problem. Concerns of how to prevent further contamination and how to reverse environmental problems that presently exist are the subjects of active investigations. The air, soil and water are susceptible to contamination by concentrations of toxic metals, organic compounds and other harmful chemicals (Baker and Brooks 1989). Most of these contaminants represent health hazards to human population. Procedures currently used to remove toxic chemicals are costly and often cause damage to soil, rendering it unusable for many years. Phytoremediation, using plants to detoxify contaminants from soils or sediments, is an excellent alternative to the current costly engineering practices (Baker et al. 1994). The potential of green plants to remove toxic metals from soil is being actively pursued by several laboratories (Cunningham and Ow 1996; Begonia et al. 1998). Plants accumulate various contaminants in their vegetative parts (Begonia et al. 1998). The purpose of this study was to determine the uptake and accumulation of Cu, Pb and Zn by *Sesbania* plants.

2. Materials and Methods

Seeds of *Sesbania* were collected from Calcasieu River in Southwestern Louisiana. Seeds were germinated following treatment with 70% sulfuric acid and 0.2% mercuric chloride. Two to three week old seedlings were placed in Hoagland media containing varying concentrations (0, 100, 500 and 1000 mg/L) of Cu [as $Cu(NO_3)_2$], Pb [as $PbSO_4$ or $Pb(NO_3)_2$] and Zn [as $ZnSO_4$]. Seedlings were grown for five weeks at 24/22°C day-night temperature regime under cool white fluorescent lamps. Each treatment had three replicates and repeated twice. At the end of each experiment, plants were removed from their respective treatments, washed thoroughly with DI water and stored at 4°C for metal analysis. In order to do a kinetic study of Cu uptake and accumulation, seedlings were grown in Hoagland media supplemented with 500 mg/L of Cu. Seedlings were collected every week for a period of four weeks and stored as described earlier. Plants were acid digested and metal contents in tissues were analyzed by Inductively Coupled Plasma Emission Spectroscopy.

3. Results and Discussion

Certain plant species have evolved mechanisms by which they can grow in soils contaminated by toxic metals (Baker et al. 1994). Their root's uptake mechanism may exclude toxic metals from the plant or may uptake the metals and sequester them in tissues without causing significant damage to them (Antonovics et al. 1971). Analysis of seedlings grown in different concentrations of Cu, Pb and Zn showed a significant increase in metal concentration in their tissues as the concentrations of metals were increased in media (Figure 1). Seedlings grown in high concentrations of metals accumulated higher amount of metals than controls. Root tissues accumulated higher concentrations (4192 μmoles/gdw) of Cu than shoots (159 μmoles/gdw) when the media was supplemented with

2000 mg/L of Cu. In some instances higher metal concentrations (>1000 mg/L) caused leaf yellowing. The kinetic study of Cu accumulation shows that the period of maximum uptake (22.5 μmoles/gdw) started in the first week of the experiment and the rate of uptake increased (34 μmoles/gdw) through the third week. This indicates that *Sesbania* can sequester toxic metals in their tissues and may have a potential role in phytoremediation of metal contaminated sites.

4. Conclusions

Sesbania can sequester Cu, Pb and Zn from media. Increasing the concentrations of metals led to an increase in the concentration of metals in plants. High concentrations of metals were toxic to seedlings, caused yellowing, and in some instances plant death. Most of the Cu was accumulated in roots.

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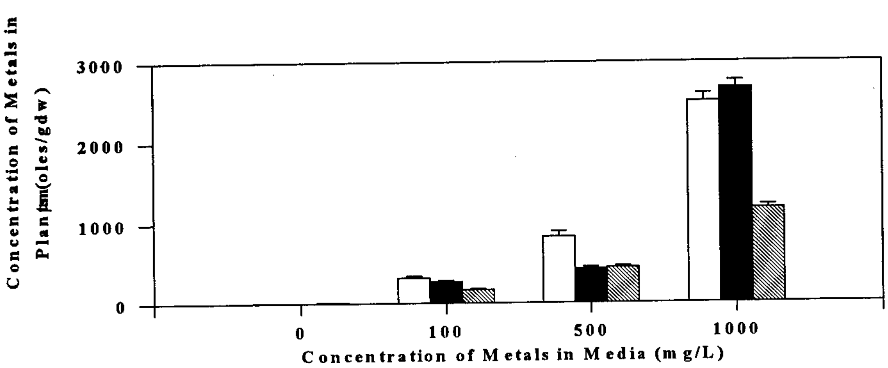

Figure 1. Concentrations of Cu (clear bar), Pb (dark bar) and Zn (hatch bar) in seedlings of *Sesbania*.

BERYLLIUM ACCUMULATION IN SOYBEAN PLANTS

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1. Introduction

Beryllium (Be) is a trace metal which is released to the environment during the combustion of fossil fuels and petroleum based products (PETERSON and GIRLING, 1981) . Due to highly toxic nature of this metal and its accumulation in the environment, the quantitative understanding of Be in a plant system is very essential. Beryllium has been shown to reduce the growth of certain plants (ROMNEY and CHILDRESS, 1965). There are few studies of Be effects on plant growth and nutrition as well as biogeochemistry of Be.. The main objective of this study was to evaluate the toxicity and distribution of Be in soybean seedlings.

2. Materials and Methods

Soybean [*Glycine max* (L.) Merr.] seeds were germinated in Perlite (tm) for 17 days until the first true leaf was fully expanded. Then seedlings were washed and transferred to 2-L opaque polyethylene pots (4 seedlings per pot) containing aerated, half strength Hoagland solution. After 7 days the Hoagland solution was changed and concentrated $BeSO_4$ solution was added to achieve treatment levels of 0, 01, 0.02, 0.04, 0.08, 0.16 and 0.32 mmol Be L^{-1} in the nutrient solution. The treatments were replicated three times and arranged in a completely randomized design. The experiment was conducted in a greenhouse with 15 hrs day and night mean temperatures 31±3°C and 23±2°C, respectively. The solution pH in each pot was adjusted to 5.2 ± 0.2 twice weekly with 0.1 *M* HCl and solution volume was adjusted daily to 2-L with deionized distilled water. After 33 days seedlings were separated into shoots and roots, rinsed in deionized distilled water, oven dried, weighed, ground and analyzed for Be, Ca, Mg, and P. The data was analyzed by using ANOVA and Duncan's New Multiple Range Test.

3. Result and Discussion

Stunting of roots and foliage became apparent within 10 d in seedlings grown in solution containing high Be concentration. The roots turned brown and foliage turned darker blue-green in color and purple streaks in the stem and the leaf size was considerably reduced. Beryllium addition significantly reduced dry weight of the seedlings by 19, 44, 58, 72, 78, and 87% in solution containing 0.01, 0.02, 0.04, 0.08, 0.16, and 0.32 mmol Be L^{-1} respectively (Table 1). The effect of Be was greater on roots than on shoot growth. The concentration of Be was substantially greater in the roots as compared to that in the shoot (Table 2). The ratio of roots:shoot Be concentration was 372 and 6 at Be concentrations in solution of 1 and 32 mg L^{-1}, respectively. This suggests that Be is relatively immobile and remained mainly in the roots. This is in line with other heavy metals studies (ADRIAÑO, 1986). Increasing concentrations of Be in the solution increased concentration of P (r^2=0.80) and decreased Ca and Mg concentrations of shoot tissue (r^2=0.78, and 0.75, respectively). It appears that phosphate metabolism was inhibited in seedlings grown in high Be solutions, thus resulting in increased accumulation of Be. The decrease in concentration of both Ca and Mg with

increased Be in solution indicates that Be either competes with cations such as Ca, and Mg for absorption sites or in some way disrupts the normal influx of Ca or Mg by damaging or clogging the absorption sites. However, this hypothesis needs further testing. Addition of Be did not significantly affect the accumulation of Ca, Mg, and P in roots.

Table 1: Dry weights of soybean shoots and roots grown in Hoagland solution with various concentrations of Be.

Be Conc..	Shoots	Roots	Total Seedling
mmol L^{-1}	g pot^{-1}		
0	8.09a	2.33a	10.42a
0.01	6.81a	1.65b	8.46b
0.02	4.63b	1.21c	5.84c
0.04	3.93bc	0.97cd	4.36d
0.08	2.24cd	0.73de	2.97e
0.16	1.71d	0.61ef	2.32ef
0.32	1.05d	0.35f	1.40f

Means followed by the same letters within each column are not significantly different (P<0.05).

Table 2: Concentrations of Be, Ca, Mg, and P in shoots and roots of soybean seedlings grown in Hoagland solution with various concentrations of Be.

Be conc.	Be Shoots	Be Roots	Ca Shoots	Ca Roots	Mg Shoots	Mg Roots	P Shoots	P Roots
mmol L^{-1}	μg^{-1}		mg g^{-1}					
0	0.0d	0.0b	19.7a	5.1ab	5.5a	6.5a	4.2c	8.1ab
0.01	1.9d	744ab	18.0ab	4.4b	5.3a	1.7b	5.3c	7.6ab
0.02	13.7d	914ab	15.6b	5.9ab	4.9ab	8.3a	6.2c	8.6ab
0.04	65.4c	1108ab	15.4b	3.9b	4.3bc	1.2b	6.5c	6.3ab
0.08	133b	1358ab	11.8c	7.3a	3.7bc	2.9b	10.4b	6.2ab
0.16	151b	1867a	9.7c	5.6ab	3.3d	6.8a	13.6	9.6a
0.32	310a	1875a	9.5c	4.5b	3.3d	1.4b	13.7a	5.8a

Means followed by the same letter within each column are not significantly different (P<0.05).

4. Conclusions

Our results suggest that while soybeans are very sensitive to Be-contaminated medium they can accumulate relatively large amounts of Be in roots. Further studies are needed to focus on the concentrations of Be (< 1 $mg^{-1}g$) that can be tolerated and/or accumulated.

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EFFECT OF LEAD APPLIED TO A BRAZILIAN OXISOL ON DRY MATTER PRODUCTION AND METAL UPTAKE BYBLACK OAT *(AVENA SPP).*

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1. Introduction

Processes of transportation and accumulation of heavy metals in soils are due to interactions with the solid phase (organic and inorganic) of the system (ALOWAY et al., 1995). These processes are very complexe, involving a lot of chemical reactions (precipitation, dissolution, complexation, oxireduction, etc.) and can be responsible for the presence of Pb in different chemical forms, resulting in its high retention, low mobility and disponibility.

The aim of this work was to study the effect of four rates of Pb on the concentration of the element in soil and in the chemical properties and shoot of black oat plants, as well as on the dry matter production.

2. Materials and Methods

The experiment was conducted in a greenhouse, using an oxisol obtained from the 0-20cm layer, which had the following chemical properties: pH ($CaCl_2$ 0.025mol.L^{-1}) 5.6; Organic matter= 21g.dm^{-3}; P resin = 51 mg.dm^{-3}; K = 0,27 $cmol_c.dm^{-3}$; Ca = 3,0 $cmol_c.dm^{-3}$; Mg = 1.3$cmol_c.dm^{-3}$; H+Al = 0,22 $cmol_c.dm^{-3}$, CEC= 6,7 $cmol_c.dm^{-3}$, and V% = 68%. Textural analysis revealed 340 g.kg^{-1} for clay, 20 g.kg^{-1} for lime, 330 g.kg^{-1} for fine sand and 310 g.kg^{-1} for coarse sand.

The experimental design was fully randomized with 4 treatments (0, 200, 400 and 600 mg Pb.kg^{-1} soil, air dried basis) and 4 replicates. The salt used for the contamination was a chloride, which was mixed to the soil with a concrete mixer. After 44 days from the installation of the rehearsal, 15 seeds of black oat were sowed in ech pot, and when they reached 5 cm height, just 4 plants were left in each pot. The fertilizer programme consisted of FeEDTA (5mL), 10 g of triple superphosfate and 15 mL of KCl (1 mol.L^{-1}). Other applications of fertilizer were done every 15 days, with 150 mg.kg^{-1} of N and 100 mg.kg^{-1} of K.

After 104 days from the installation of the experiment, plants were cut just above the soil surface, air dried (60-70° C) milled to 40 mesh and analysed for Pb content in nitric perchloric extract by ICP. Soil samples were obtained, air dried and Pb was determined in the Melich-1 extract.

3.Results and Discussion

According to Figure 1, Pb in the shoot of black oat plants increases with the rates of applied Pb in a linear way. The maximum Pb content observed was about 5.18 mg per plant, which is a high level, concerning the fact that this is the maximum concentration allowed for 1 kg of dry matter.

Figure 2 shows Pb contents in the soil, which had a large increase until 200 mg.kg^{-1}, and stabilized after this point. This can be due to interactions between lead and chemical species of the solid phase, as well with organic matter.

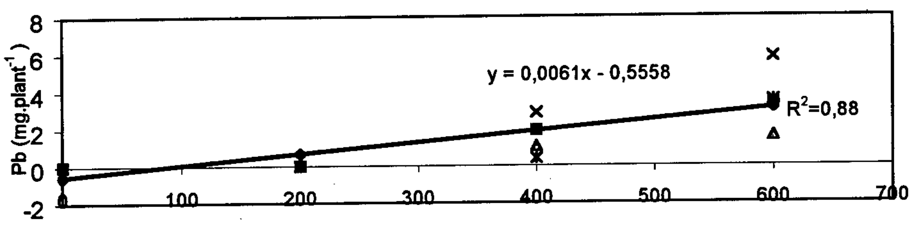

Figure 1. Effect of increasing rates of Pb applied on Pb uptake by black oat plants (aerial part).

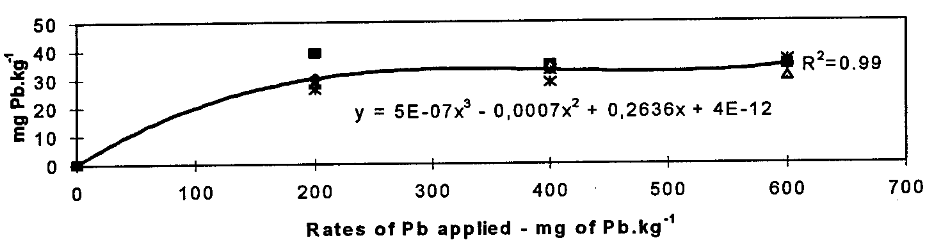

Figure 2. Effect of increasing rates of Pb on soil Pb contents (extracted by the Melich-1 method)

4. Conclusions

Pb levels had no effect on dry matter production, but resulted in increases on Pb contents at the shoots in a linear way. Pb applied increased the soil Pb contents as well.

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CADMIUM AVAILABILITY IN AGRICULTURAL SOILS ASSESSED BY THE ISOTOPIC EXCHANGE KINETIC TECHNIQUE.

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1.Introduction

Total cadmium (Cd) content in the soil and the soil solution, as well as Cd concentration extracted by chemical agents are usually insufficient data to predict the total Cd available to the plant. Frequently, these data depend on soil properties and on the chemical agent used. Moreover, the pool of available element is not determined. The isotope exchange kinetics in soil-solution allows to characterize this pool for P, K, Zn and Ni (ECHEVARRIA et al., 1997; FROSSARD and SINAJ, 1997). This paper shows applications and limits of the isotope exchange kinetic method to assess the Cd transfer from the solid soil phase to the soil solution in the agricultural soils.

2. Materials and Methods

Soil samples were collected in the 0-0.25 m surface-ploughed layer of agricultural fields located at Loiches, Chassigny, Nitry and Demay, Yonne district, Burgundy, France (MENCH et al., 1997). The isotope exchange kinetic method was adapted from FARDEAU (1993) who study the soil-solution transfer of phosphate. When a radioactive tracer (**R**) is added to a soil solution at a steaty state equilibrium (**C_{Cd}**), the radioactivity (**r**) in solution decreases with the time (**t** in minutes). The decreasing radioactivity in the solution is ascribed to a homoionic exchange between radioactive ions in the solution with ions at the solid soil phase. The amount of isotopically exchangeable Cd (**E**) is obtained by $E = V/M \times C_{Cd}/(r/R)$ (equation 1) with $V/M = 10$.
15 g of soil (2 mm sieved air-dried soil) was shaken with 149.1 ml of distilled water + 0.075 ml of micr-o-protect® for 17 hours to obtain a suspension with an equilibrated Cd concentration. At t = 0 minute, 0.9 ml of ^{109}Cd with an amount of radioactivity (≈ 0.44 MBq) was introduced to the stirred suspension, sampled with a seringe after 1, 4, 10, 40, 100 minutes and filtered (0.2 µm). The radioactivity in the solution was measured by liquid scintillation. The Cd concentration in the solution was measured by Graphit Furnace Atomic Absorption Spectrometry (GFAAS) at the 10 and 100 minutes.

3. Results and Discussion

Soil characteristics are listed in Table 1. C_{Cd} values are maintained at a steaty state equilibrium between 10 and 100 minutes (Table 1), but concentrations are low (mean of 0.25 µg kg^{-1}). The working limit with GFAAS is 0.2 µg kg^{-1} (calculated as 10 times the detection limit). In contrast to results obtained for P, Ni, Zn and As, r_t/R decreases immediately during the first minute for the 4 soils. r_t/R value is maintained between 1 to 100 minutes (fig 1), whereas in polluted soils, r_t/R decreases as a function of time (SAPPIN-DIDIER unpublished). After 1 minute, 99.7 to 99 % of ^{109}Cd is exchanged with the Cd into the solid soil phase. The exchangeable Cd (**E** value, eq. 1) is not a function of time (Figure 1). The E pool contains both Cd in soil solution (C_{Cd}) and quickly exchangeable Cd sorbed on the solid phase. C_{Cd} is very small compared to exchangeable Cd. A K_d index, $K_d = (E - 10\ C_{Cd}) / 10\ C_{Cd}$ (equation 2), has been calculed to determine the capacity of the

solid phase to supply the Cd in the soil solution. K_d of the Demay soil is lower than that of the three other soils. For these agricultural soils, Cd can be distributed among two compartments : the first one being an E pool of quickly exchangeable Cd and the second one being a pool of Cd that cannot be exchanged (noted NE), corresponding to the difference between Cd total content and exchangeable Cd (E). The NE compartment represents 70% of the total Cd content for the Demay soil, 34% and 47 % for the Loiches and Chassigny soils, and 20 % for the Nitry soil. The study presents a physico-chemical characterization of Cd availability and non-availability in agricultural soils. Further complementary experiments must be done to shown the relationship between plant-available Cd and its exchangeability, and to determine the speciation of Cd in soil solution (Cd^{++}). The Cd speciation analysis in the soil solution may improve the Cd availability assessment.

Table 1 : Soil characteristics, total Cd content in the soils (µg kg^{-1}), values of C_{Cd} at 10 and 100 minutes (µg l^{-1}) (with their 95% confidence interval) (* : no difference at the 5% level – Newman-Keuls test) (n = 2) and soil capacity to supply the soil solution (K_d).

SOIL	FAO-UNESCO (1988)	Clay %	Silt %	O.M. %	pH water	Total Cd (µg kg^{-1})	* C_{Cd}(µg l^{-1}) 10-100 minutes	K_d
Loiches	Stagnic Luvisols	30	62	2.4	6.2	730	0.31 (0.04)	154
Chassigny	Stagnic Luvisols	31	54	2.9	6.7	430	0.23 (0.08)	99
Nitry	Chromic Luvisol	39	59	2.2	5.7	370	0.27 (0.04)	110
Demay	Rendzic Leptosols	47	37	3.6	8.1	2020	0.20 (0.01)	317

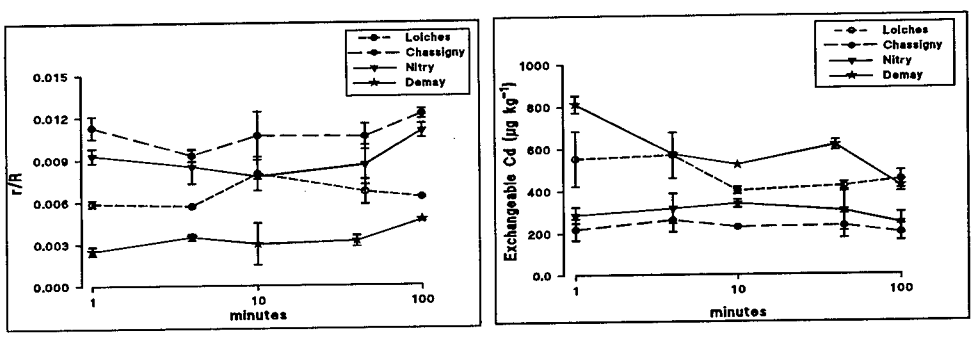

Figure 1 : Kinetics of r/R values and exchangeable Cd (µg.kg^{-1}) (E) for Loiches, Chassigny Nitry, Demay soils.

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EFFECT OF LONG-TERM APPLICATION OF WASTEWATER ON BIOAVAILABILITY OF TRACE ELEMENTS

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1. Introduction

The Gabal El Asfar Old Farm is a government operated fruit plantation situated north of Cairo in the Eastern Desert. The farm has been irrigated with sewage for more than 80 years and there are concerns about soil, water and crop contamination at the site and also about potentially toxic heavy metals. The site provides a possible model of the potential long-term effects of heavy metals on crops for sewage sludge-treated soils in Egypt.

2. Materials and Methods

The heavy metal contents of citrus leaves and fruit (orange and mandarin) and total and DTPA extractable concentrations in soils were measured in samples collected from different areas of Gabal El Asfar Old Farm during two surveys of the site in 1997.

3. Results and Discussion

Total and DTPA concentrations of heavy metals in the surveyed soils showed significant enrichment by long-term irrigation with sewage effluent. For example, the maximum total concentrations of Zn and Cu were 530 and 366 mg kg^{-1}, respectively, representing a potential risk to crop yields (Table 1). The maximum Cd concentration detected was 9 mg kg^{-1} and Cd may be a potential risk to the human food chain from uptake into staple crops grown at the farm. The concentrations of DTPA extractable metals were also increased by sewage application and were significantly correlated ($P<0.001$) with the total contents of Zn ($r=0.81$), Cu ($r=0.89$), Ni ($r=0.88$), Cd ($r=0.73$) and Pb ($r=0.62$) (Figure 1a). However, crop analysis showed no relationships were apparent between tissue content (Table 2) and the corresponding total and DTPA extractable concentrations in soil for the elements determined (Table 3 and Figure 1b). Leaf Zn and Cu concentrations were in the low (16-24 and 3.6-4.9 mg kg^{-1}, respectively) to optimum (25-100 and 5-16 mg kg^{-1}, respectively) ranges for citrus and the other heavy metals were within normal ranges. Whilst DTPA is widely used in nutrient diagnosis assessment, it has not provided a reliable indication of the bioavailability of potentially toxic elements to citrus in reclaimed desert soil.

4. Conclusions

This study of long-term contamination of reclaimed desert soil with heavy metals demonstrates the minimal risk to crop quality, yield and human health from potentially toxic elements applied to soil in sewage sludge for desert reclamation and fruit production.

Table 1 Total heavy metal content (mg kg^{-1}) of soil at Gabal El Asfar Old Farm

Element	Survey 1			Survey 2		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Zn	180	530	331	32	143	95
Cu	50	117	84	7	366	67
Ni	1	51	22	10	92	45
Cd	1	9	3	0.2	4.6	1.6
Pb	5	70	23	16	290	70
Cr	80	230	154	2	376	89

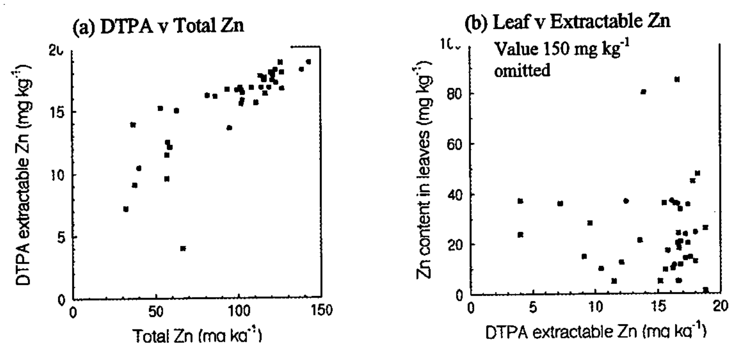

Figure 1 Relationships between (a) total and extractable Zn in soil and (b) extractable Zn in soil and Zn content of citrus leaves at Gabal El Asfar Old Farm (Survey 2)

Table 2 Heavy metal content (mg kg^{-1}) of citrus at Gabal El Asfar Old Farm

Element	Fruit (Survey 1)			Leaves (Survey 2)		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Zn	0.4	2.6	1.1	1.2	150	27.8
Cu	0.1	0.5	0.3	1.0	9.4	3.8
Ni	0.1	1.2	0.4	0.8	10.0	4.3
Cd	0.02	0.1	0.07	<0.1	0.2	0.1
Pb	0.2	2.1	1.0	0.5	30.0	12.4
Cr	0.4	3.6	2.0	0.2	4.1	2.0

Table 3 Correlation coefficients (r) of relationships between metal concentrations in soil and citrus at Gabal El Asfar Old Farm

Element	Fruit		Leaves	
	Total	DTPA	Total	DTPA
Zn	-0.11ns	0.03ns	-0.01ns	0.05ns
Cu	0.27ns	-0.03ns	0.36ns	0.24ns
Ni	0.06ns	-0.35ns	0.38ns	0.18ns
Cd	0.39ns	0.35ns	0.35ns	0.47ns
Pb	0.24ns	-0.24ns	-0.10ns	0.04ns
Cr	0.09ns	0.01ns	0.01ns	-0.05ns

ns, not significant at P=0.05

PHYTOCHELATINS IN RELATION TO GRAIN CADMIUM ACCUMULATION IN WHEAT

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This is a new project with the aim to investigate how cadmium (Cd) is translocated to the wheat grain after uptake by the root, and the importance of phytochelatins (PCs) for this translocation.

Cadmium is strongly toxic to the plant and various physiological and biochemical processes are severely affected by cadmium, *e.g.* photosynthesis, root growth and storage proteins (Larsson et al., 1998, Chugh and Saharan, 1991). Cadmium also induce the synthesis of stress proteins, like PR proteins (Stolt et al., in preparation). The mechanisms behind tolerance to Cd can be divided into two groups (i) the ability to prevent excessive uptake of metal ions; (ii) the ability to handle high levels of heavy metals in the tissue (de Knecht, 1994). One possible way to detoxify Cd is to produce PCs which can bind and thus neutralize the deleterious effects of the metal.

Cadmium also has impact on the quality of cereal grains for feed and food. This is a serious problem and some Swedish grown bread wheat exceed the FAO/WHO (1993) recommended maximum level of 100 µg Cd/kg dw grain (Landner et al., 1995). There is, however, a genotypic variation in wheat grain Cd accumulation (approx. 40-250 µg Cd/kg dw for the cultivars used in this study). This variation is related to differences in the Cd transport in the plant, especially from root to shoot (Lundborg and Ostreus unpublished).

The intention of this study is to investigate if and how PCs are involved in the control of Cd transport in the wheat plant. PCs are non-protein thiols with the general structure $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ where n=2-11 (Grill et al., 1987). The affinity to Cd is strong due to the richness of cystein in the PC molecule (Steffens, 1990). Wheat respond to heavy metals not only by accumulating PC-Gly, but also by accumulating PC-Ser (Klapheck et al., 1992).

The aim of the study is to

- characterize PCs in different organs of wheat
- investigate the pattern of induction and synthesis of PCs
- relate the presence of PCs to Cd transport and Cd grain accumulation-describe genotypic variation in Cd transport and PC pattern.

The role of PCs for the Cd transport in wheat will be discussed, as well as possible relationships between Cd translocation and Cd tolerance in the wheat plant.

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EXTRACTABILITY AND PLANT-AVAILABILITY OF SOIL AS AND CD AS INFLUENCED BY SOIL PROPERTIES

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1. Introduction

The plant availability of soil elements is affected by wide complex of physical - chemical properties of soil, chemical properties of individual elements and plant species. Soil pH, clay and oxides content, and structure of organic matter are the most frequently discussed. In our experiment, the influence of main soil properties on plant-availability and/or extractability of soil arsenic and cadmium was evaluated. From the large scale of soil extraction agents used, mild extractants were preferred because of their similarity to soil solution.

2. Materials and Methods

35 soil samples covering the area of Czech Republic were tested in this experiment. The soils were characterized by pH_{KCl} in the range of 3.6 - 7.3, cation exchange capacity (CEC) of 8.9 - 29.2 meq.100 g^{-1}, content of organic carbon (C_{ox}) of 0.7 - 6.7 %, content of clay particles of 10 - 48 %, total arsenic concentration of 4.4 - 352 $mg.kg^{-1}$ and total cadmium concentration of 0.1 - 19.4 $mg.kg^{-1}$. In pot experiment, spinach (var. Estivato) and radish (var. Duo) were planted at these soils. 1 $mol.l^{-1}$ NH_4NO_3 (1:2.5), 0.1 $mol.l^{-1}$ $NaNO_3$ (1:2.5) and 0.01 $mol.l^{-1}$ $CaCl_2$ (1:10) solutions were applied for the extraction of the soil samples. Atomic absorption spectrometry was used for the determination of As and Cd in both plant samples and soil extracts. For an evaluation of plant-availability of elements, transfer factor (T.F.) given as ratio of total element concentration in plant and in soil was used.

3. Results and Discussion

Transfer factors of As and Cd showed that the plant-availability of cadmium (T.F. in the range of 0.12 - 29.9) is significantly higher as compared to arsenic (T.F. in the range of 0.002 - 0.30). For both elements, the plant availability increased in the order radish roots < radish leaves < spinach leaves. Cd availability was predominantly affected by soil pH (increasing soil acidity led to significantly higher cumulation of Cd in plants, especially in spinach). The other soil parameters did not significantly correlate with Cd accumulation in plants. In the case of arsenic, increasing levels of CEC, C_{ox} and clay particles slightly suppressed the arsenic cumulation in plants. However, the extremely high content of organic matter (C_{ox} > 4 %) led to better As availability compared to common levels of C_{ox} (1-2 %).

Concerning extractability of elements from soils by NH_4NO_3, $NaNO_3$ and $CaCl_2$ solutions, the conclusion was similar to the above mentioned plant-availability. In the case of arsenic the extraction yield did not exceed 1.5 % of total soil arsenic concentration and decreased in the order $CaCl_2$ > $NaNO_3$ > NH_4NO_3. Amount of available Cd represented in some samples almost 50 % of total soil Cd and decreased in the order $CaCl_2$ > NH_4NO_3 > $NaNO_3$. An example of investigated relationships among elements and soil parameters is shown in Figure 1. The important role of soil pH for Cd extractability is evident as well as the importance of C_{ox} in the case of As. All the three extracting agents tested can be evaluated similarly. Both transfer factors and extractable element

concentrations seemed to be related to individual soil parameters by similar way. From this reason the correlations given in Figure 2 can be suggested as acceptable.

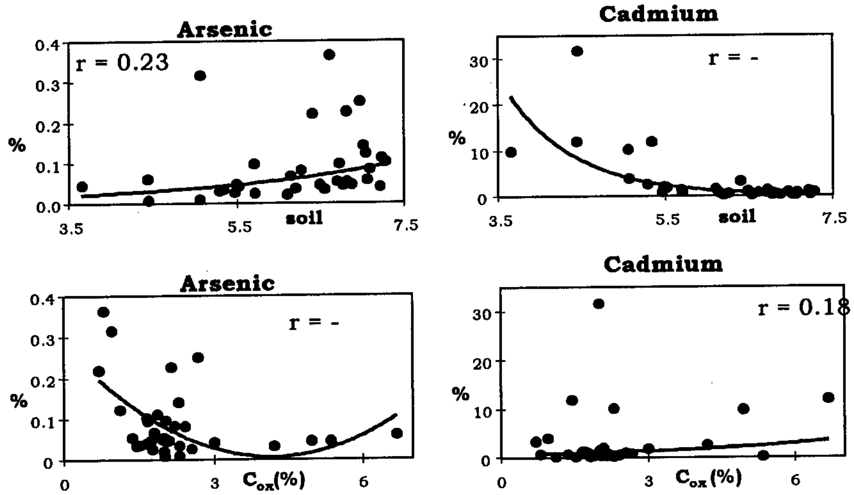

Figure 1: The influence of pH and content of organic carbon in soil on 1 mol.l^{-1} NH_4NO_3 extractable concentrations of As and Cd (% of total element conc.). Correlation coefficients marked by * were statistically significant at α = 0.05.

4. Conclusions

Compared to easily available cadmium, arsenic was tightly bound to individual soil components and its availability seemed to be more significantly related to the complex of physical-chemical properties of individual soils. Mild soil extraction procedures (NH_4NO_3, $NaNO_3$ and $CaCl_2$ solutions) were able to release soil element fractions related to the individual soil parameters similarly as the plant-available fractions of these elements.

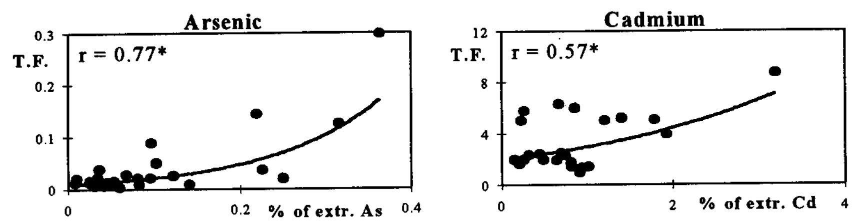

Figure 2: The relationships between transfer factors for spinach and 1 mol.l^{-1} NH_4NO_3 extractable concentrations of element (% of total concentration). Correlation coefficients marked by * were statistically significant at α = 0.05.

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INFLUENCE OF SOIL PH ON CADMIUM UPTAKE BY TOBACCO

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1. Introduction

Cadmium (Cd) is a non essential, potentially toxic element accumulated by tobacco which concentrates it mainly in leaves from which is transferred in tobacco smoke. Cadmium is accumulated in kidneys of smokers which have twice the amount of Cd in their kidneys than in non-smokers (Wagner, 1993). There is evidence that Cd is increasing in the agricultural environment mainly because of phosphatic fertilizer use, sewage sludge dispersal or atmospheric deposition. Soil pH is one of the most important factors regulating Cd uptake by plants. The purpose of the present study was to investigate the influence of soil pH in Cd uptake by tobacco with final goal to use this soil factor in reducing Cd uptake by tobacco.

2. Materials and Methods

A pot experiment was conducted with an acid soil classified as Typic Haploxeralf coming from Pieria, Greece. Five kg sub-samples were limed to obtain a pH range from 5.28 to 7.01 and contaminated with 20 mg kg^{-1} Cd that was applied as $CdCl_2$. Finally, six treatments were obtained each replicated 3 times and were arranged in a complete randomised block design. Seeds of *Nicotiana tabacum* L. Samsun 53, were sown in a seedbed made of sand-manure mixture and when the seedlings became 15 cm tall were transplanted one per pot in plastic pots, and maintained under natural conditions. During the growing season which lasted about three months, plants were irrigated with deionised water. Leaves were harvested four times according to their maturation. Leaves after the proper preparation, were analyzed for Cd (Mills and Jones, 1996). Soil samples were selected at the end of the growing period and analyzed for pH and Cd using the DTPA method. In the same soil samples Cd was fractionated into exchangeable ($CdKNO_3$), organic matter associated (CdNaOH), carbonate associated (CdEDTA), and residual Cd ($CdHNO_3$) according to the procedure of Emmerich et al., (1984). In all the cases Cd was determined using atomic absorption spectrophotometry.

3. Results and Discussion

Soil pH influence on tobacco yield as well as on total Cd uptake by tobacco and on soil Cd forms is presented in Table 1. From the data of Table 1 it is obvious that soil pH increase, positively affected tobacco yield, probably because of the decrease of Cd uptake. From the Cd forms determined $CdKNO_3$ as well as $CdHNO_3$ concentrations were significantly decreased, while CdEDTA was significantly increased. CdNaOH did not show a clear trend in relation to soil pH. Cadmium extracted by DTPA was significantly decreased in the pH values greater than 6.87. Graphical presentations of the relationships between tobacco yield and Cd uptake with $CdKNO_3$ and $CdKNO_3$ and Cd uptake with soil pH are given in the Figure 1.

4. Conclusions

Soil pH increase due to liming, substantially decreases Cd uptake by tobacco decreasing exchangeable Cd ($CdKNO_3$) which is a very good indicator of Cd availability to tobacco.

Table 1. Influence of soil pH on tobacco yield, Cd uptake by tobacco and concentration of CdDTPA and heavy metal fractions

PH	Yield	Cd uptake	CdDTPA	$CdKNO_3$	CdNaOH	CdEDTA	$CdHNO_3$
	g/pot	µg Cd/pot			mg kg^{-1} soil		
5.28a*	7.5a	1434c	11.53b	5.76b	0.34ab	6.08a	11.53b
5.60b	7.9ab	703b	11.18b	3.03ab	0.32a	8.86ab	11.18b
6.37c	9.7c	407a	12.87b	1.51ab	0.40ab	15.70c	12.87b
6.57d	7.5a	304a	12.78b	3.86ab	0.27a	13.22bc	12.77b
6.87e	8.5abc	360a	12.53b	0.73a	0.46b	17.79c	2.13a
7.01f	9.5bc	345a	6.98a	0.47a	0.30a	8.29ab	0.93a

*Numbers in the same column followed by different letters differ significantly (P<0.05) according to the LSD test

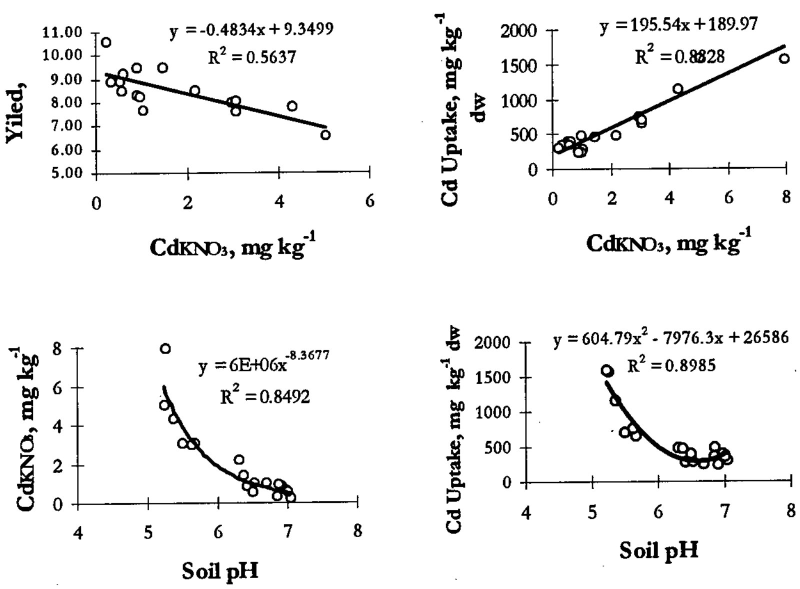

Figure 1. Relationships between $CdKNO_3$ with tobacco yield, and Cd uptake and soil pH with $CdKNO_3$ and Cd uptake

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